# SYNTHESIS AND PHARMACOLOGICAL SCREENING OF SOME ANALOGUE HAVING ARYL/HETEROARYL NUCLEUS HAVING ALKYLAMINOHYDROXYPROPOXY SIDE CHAIN

**A Thesis**

**Submitted in the partial fulfillment of**

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**In**
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(Faculty of Medicine)

**Saurashtra University, Rajkot.**


**By**

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# *CERTIFICATE*

This is to certify that the thesis entitled **"Synthesis and Pharmacological Screening of Some Analogue Having Aryl/Heteroaryl Nucleus Having Alkylaminohydroxypropoxy Side Chain"** represents bonafide work of **MR. BHAVESH LIMBABHAI THUMBER,** carried out under my guidance and supervision. The work mentioned in this thesis was carried out at R. K. College of Pharmacy, Rajkot during the period of years 2007-2011. The work is up to my satisfaction.

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## ***DECLARATION BY THE CANDIDATE***

I, hereby, declare that Saurashtra University, Rajkot shall have the right to preserve, use and disseminate this thesis, in print or electronic format, for academic/research purpose.

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## Statement Under Ordinance Ph. D. 7 of Saurashtra University

The contents of this thesis are my own work, carried out under supervision of Dr. T. R. Desai and Dr. Y. T. Naliapara. It leads to some contribution in pharmacy, supported by necessary references.

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# ***DECLARATION***

I hereby declare that the thesis entitled "**Synthesis and Pharmacological Screening of Some Analogue Having Aryl/Heteroaryl Nucleus Having Alkylaminohydroxypropoxy Side Chain"** is a bonafide research work, carried out by me, under the guidance of Dr. T. R. Desai and Dr. Y. T. Naliapara. This work is original and has not been submitted in part or full for any degree/diploma to other University**.**

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# DEDICATED

# TO

# MY

# BELOVED

# FAMILY

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"I am the Vedic ritual, I am the sacrifice, I am the offering to the departed; I am the herbage and foodgrains; I am the sacred formula, I am the clarified butter, I am the sacred fire, and I am verily the act of offering oblations into the fire." (Shreemad Bhagvad Geeta 9/16)

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# CONTENTS

## ***List of Tables***

## ***LIST OF FIGURES***

## ***List of abbreviations***

| | |
|---|---|
| LIMC | Low and middle-income countries |
| CVDs | Cardiovascular diseases |
| WHO | World Health Organization |
| ACE | Angiotensin converting enzyme |
| ASA | Acetylsalicylic acid |
| PVD | Peripheral vascular disease |
| CHD | Coronary heart disease |
| FABP | Fatty acid-binding protein |
| EDDA | Ethylenediammonium diacetate |
| THF | Tetrahydrofuran |
| MOM | Methoxymethyl ether |
| IPA | Isopropyl alcohol |
| NA | Noradrenalin |
| M.P. | Melting Point |
| B.P. | Boiling Point |
| RBF | Round Bottom Flask |
| RT | Room Temperature |
| TLC | Thin Layer Chromatography |
| SEM | Standard Error Mean |
| NMR | Nuclear Magnetic Resonance |
| GC | Gas Chromatography |
| FTIR | Fourier Transform Infra Red |
| ANOVA | Analysis of Variance |

# 1. ABSTRACT

There is no health system in place to deliver the affordable drugs that can treat and prevent the disease burden for those in need. Cardiovascular disease is ravaging India and China. Unfortunately, though, the people of such low- and middle-income countries (LMIC) are not able to access preventive treatments widely available in the West. Cardiovascular disease was the leading cause of death globally in 2005 with more than 80 per cent of these deaths occurring in LMIC. In China, stroke, chronic obstructive airways diseases, cancer and heart disease are the four highest contributors to the country's total disease_burden almost half of these are due to cardiovascular disease. Cardiovascular drugs include antihypertensives, antihyperlipidemics, vasodilators, anticoagulants, diuretics and other Agents. [1a] According to World Health Report 2002, cardiovascular diseases (CVDs) will be the largest cause of death and disability by 2020 in India (WHO 2004). [8]

Hypertension damages blood vessels in kidney, heart, and brain and leads to an increased incidence of renal failure, coronary disease, cardiac failure, and stroke. Hypertension is a major risk factor for two distinct kinds of vascular problems: Complications of atherosclerosis including myocardial and cerebral infraction as well as complications of small vessel disease including renal failure, intracerebral hemorrhage and lacunar infractions. [17]

Therapy using the antihypertensive agents evolved rapidly between 1950 and 1960. During that time a number of empiric discoveries were made that resulted in the marketing of the drugs for the treatment and control of hypertensive diseases. The conventional treatment for the hypertension includes various drugs from the categories of diuretics, β-blockers, calcium channel blockers, ACE Inhibitors, Type-1 angiotensin II receptor antagonists, $\alpha_1$ Adrenoceptor antagonists, vasodilators etc. [19]

Antagonism of β-adrenergic receptors affects the regulation of the circulation through a number of mechanisms, including a reduction in myocardial contractility, heart rate, and cardiac output. An important consequence of using β - adrenergic receptors is blockade of the β

receptors of the juxtaglomerular complex, reducing renin secretion and thereby diminishing production of circulating angiotensin II. This action likely contributes to the antihypertensive action of this class of drugs, in concert with the cardiac effects.

Propranolol is the proto type agent for class of β - adregernic bloking agents. Propranolol has equal affinity for $\beta_1$ and $\beta_2$ adrenergic receptors; thus, it is a nonselective β adrenergic receptor antagonist. Propranolol is a pure antagonist, lacks intrinsic sympathomimetic activity. [21] Because all β adrenergic receptor antagonists are effective antihypertensive agents and (+)-propranolol, the inactive isomer that has little β-adrenergic receptor blocking activity, has no effect on blood pressure, the antihypertensive therapeutic effect of these agents is undoubtedly related to receptor blockade.[22]

In medicinal chemistry, the chemist attempts to design and synthesize a medicine or a pharmaceutical agent which will benefit humanity. Recently, several clinical trials have demonstrated that beta-blockers remarkably reduced mortality in patients with moderate heart failure as well as improved the quality of life and sense of well-being by reducing hospitalizations and arrhythmias. [24] This name originates from the presences of an $–OCH_2$-group located between a substituted aromatic ring and an ethylamino side chain of pronethalol (1). The aromatic ring and substituents are the primary determinants of β antagonist selectivity. Propranolol (2), nadolol (3), timolol (4) and pindolol (5) are non-specific β blokers whereas para position substitutions on the aromatic rings of metoprolol (6), atenolol (7), esmolol (8), acebutolol (9) both confer $\beta_1$ antagonist selectivity.[25]

The concept of alkyl aminohydroxypropoxy (10) side chain derivatives is understood by class of β – adrenergic blocking agents. Researchers who were studying the effects of aryl ring and aryl substitution in the molecule tried to modify the ethanolamine chain itself by inter alia, the introduction of linking group between aryl ring and ethanolamine chain. After lots of linking groups tried, the best linking group comes out to be oxymethylene. It is first analog, propranolol which is the most widely used β - blocker now and is 10 to 20 times potent than its parent compound pronethalol.[26]

**Thromboembolism** is the combination of thrombosis and its main complication, embolism. When a thrombus occupies more than 75% of surface area of the lumen of an artery, blood flow to the tissue supplied is reduced enough to cause symptoms because of decreased oxygen (hypoxia) and accumulation of metabolic products like lactic acid. More than 90% obstruction can result in anoxia, the complete deprivation of oxygen, and infarction, a mode of cell death. The symptoms of a thromboembolism depend on the organ or blood vessel that has lost blood supply. Blood clots in an arm or leg may cause pain, swelling, and increased temperature in the affected area. A clot that travels to the lung is called a pulmonary embolus. This condition can cause: chest pain, shortness of breath, rapid heartbeat, known as tachycardia, fainting or death. If a blood clot is formed in the heart, it can travel to almost any organ in the body. This could cause a stroke, which is a type of damage to the brain from lack of blood circulation. In other cases, damage may be done to an arm or leg, or a heart attack or kidney damage may occur. Other areas of the body can also be affected.

An anticoagulant is a substance that prevents coagulation; that is, it stops blood from clotting. Anticoagulants were introduced into medical practice more than three decades ago. Extensive use of these drugs in the prevention and treatment of thromboembolic disease has made them one of the most widely used classes of pharmacological agents.

Anticoagulant drugs include:

a) Heparin and derivative substances e.g. Low molecular weight heparin

b) Vitamin K antagonists e.g. Warfarin

Warfarin and related 4-hydroxycoumarin-containing molecules decrease blood coagulation by inhibiting vitamin K epoxide reductase, an enzyme that recycles oxidized vitamin K to its reduced form after it has participated in the carboxylation of several blood coagulation proteins, mainly prothrombin and factor VII.

The story of the coumarin anticoagulants generally is traced back to the early 1920s, when the "sweet clover disease" showed up almost simultaneously in North Dakota and in Alberta, Canada. This new malady of cattle involving fatal bleeding was traced to stacks of sweet clover hay.[29] Thousands of synthetic equivalents were prepared in 1940. Since 1940, hundreds of 4-hydroxycoumarin-based compounds have been prepared and tested for anticoagulant activity, among these; Warfarin quickly became the leader in clinical anticoagulant therapy. Coumarin has a characteristic hay-like, sweet aromatic creamy odor with certain nutty shadings, much used in synthetic form as a fragrance chemical for various products like toothpastes, antiperspirant deodorants, bath products, body lotions, face creams, fragrance creams, hair sprays, shampoos, shower gels and toilet soaps. Coumarin has been used in detergents as a brightener or bleaching agent. It is used as an odor-enhancer to achieve a long-lasting effect when combined with natural essential oils such as lavender, citrus, rosemary and oak moss.

Marshall et al. (1987) stated that coumarin has been recommended for treatment of a number of clinical conditions, including high protein edema and brucellosis. Kontogiorgis *et al.* (2005) stated the anti inflammatory and antioxidant activity of novel coumarin derivatives. Anamik *et al.* (2005) synthesized series 4-hydroxy derivatives and screened them in vitro for anti-HIV activity against HIV-1(IIIB) and HIV-2(ROD) virus strains. Compound (41) was found to be the most active.[112] Shen *et al.* (2010) worked on hydroxycoumarin derivatives as novel and potent α-Glucosidase Inhibitors, hence use as insecticide.

The main complications of hypertension, i.e. coronary heart disease, ischemic strokes and peripheral vascular disease (PVD), are usually related to thrombosis. Increasing evidence also suggests that hypertension fulfils the components of Virchow's triad, thus conferring a prothrombic or hypercoagulable state, as evident by abnormalities of haemostasis, platelets and endothelial function. It therefore seems plausible that use of antithrombotic therapy may help prevent these thrombosis-related complications of hypertension. Indeed, hypertensive patients with an estimated 10-year CHD risk > or = 15% will have their cardiovascular risk reduced by 25% using antihypertensive treatment, but the addition of aspirin further reduces

major cardiovascular events by 15%. Recent guidelines recommend the use of aspirin 75 mg daily for hypertensive patients who have no contraindication to aspirin.[35] In addition it should be recognized that some of the complications related to elevated blood pressure, heart failure or atrial fibrillation, are themselves associated with thromboembolism.[37] Increasing evidence also points towards a prothrombic or hypercoagulable state conferred by the presence of elevated blood pressure, as evident by abnormalities of coagulation, platelets and endothelial function in such patients.[38-39]

It therefore seems plausible that use of antithrombotic therapy may be of particular benefit in preventing the thrombosis-related complications of elevated blood pressure.[40] The antithrombotic agent, acetylsalicylic acid (ASA), is established as an effective agent for secondary prevention in patients with proven occlusive vascular disease.[41] However, it is not recommended for primary prevention and it is unclear whether it has a role in patients with an increased risk of thrombotic complications such as those with elevated blood pressure. Warfarin has also been found to be useful as thromboprophylaxis in patients with elevated blood pressure and atrial fibrillation; however, if blood pressures remain uncontrolled, such therapy carries significant risk, especially from intracranial hemorrhage.

Thus, since hypertension and thrombo-embolism are intricately involved in pathophysiological changes and progression of cardio-vascular diseases; antihypertensive drugs like propranolol and anticoagulant drugs like Warfarin are frequently, combined in management of some types of hypertension. However, in clinical practice, it has been noted that prescribing, one drug with two different activities, improves patient compliance to the treatment instead of two drugs with two different activities resulting in better clinical outcome. Therefore, in the present project, we attempted to synthesize a single drug molecule with antihypertensive activity of propranolol and anticoagulant activity of Warfarin (Coumadin).

To achieve this, we attached alkylaminohydroxypropoxy side chain (responsible for antihypertensive activity of propranolol) to 4-Hydroxy coumarin nucleus (responsible for anticoagulant activity of Warfarin). Several resulting molecules were then, subjected to pharmacological evaluation, for both types of activities, to assess structure activity relationship and to ascertain the lead molecule.

In our project, for synthesis of substituted 4-hydroxy coumarin nucleus, we used phenol, m-cresol and p-cresol as starting materials. Thus, we synthesized 3 basic coumarin nucleus viz. 4-hyddroxy coumarin, 7-methyl-4-hydroxy coumarin and 8-methyl-4-hydroxy coumarin nucleus, respectively. Each nucleus was then, reacted with epichlorhydrin to form three varieties of epoxy derivatives. Finally, each epoxy derivative was reacted with 10 different amines to form 10 different coumarin molecules. All resulting 30 compounds, were then, converted to hydrochloride salts, by reacting them with dry hydrochloric acid gas.

Bose *et al* synthesized 4-hydroxy coumarins by heating diaryl malonates with equimolor moles of corresponding malonic acid in the presence of about anhydrous $ZnCl_2$ and $POCl_3$. We attempted to carry out this reaction at $70^0$C for 12-15 hours, but it was observed that maximum yield was obtained only after 16 hours. It was also noted that on acidification of sodium bicarbonate filtrate, at neutral point, the product precipitated out. The yield was increased, if acidified slurry was kept overnight. Hence, this modified version of reaction was adopted, for synthesis of all other coumarin derivatives, in our project. Our attempts to carry out reactions of other phenols like α-napthol, β-napthol and hydroxyl benzoic acid were unsuccessful. When 4-hydroxy coumarin derivative was treated with epichlorhydrin in presence of base, under suitable condition, resulted in formation of the epoxy derivative. To obtain solid product, the epoxy resin was completely separated from reaction mixture, by using mother solvent i.e. toluene. Then semisolid mass was obtained. Semisolid mass was obtained only, when the solvent or unreacted amine was distilled out. When this product was dissolved in 1:5 mixture of DMF + dioxane then poured on to ice + water mixture, a free product (base) in aqueous medium obtained. But when it was filtered, it soon converted in to brown semisolid mass.

Synthesized compounds conformed to the spectral analysis. Infra Red Spectra were taken on Shimadzu FTIR-8400 Spectrometer, using KBr Pellet method. The characteristic peak of carbonyl group in coumarin moiety was observed at 1700-1722 $cm^{-1}$ frequency, while C-O streching of ring skeleton was observed at 1160-1125 $cm^{-1}$ frequency. The N-H streching of secondary amine gave a broad peak, between 3350-3300 $cm^{-1}$ frequency. The C-N streching was observed at 1250-1235 $cm^{-1}$ frequency. The –OH bending was observed at 1380-1310 $cm^{-1}$ frequency. Other frequencies, observed due to ring skeleton, were around 1600-1450 $cm^{-1}$ frequency of C=C stretching and 2900-3000 $cm^{-1}$ frequency, was due to C-H stretching. Further conformation of the molecular structure was evaluated by mass spectra. The mass spectrum of compounds were recorded by Shimadzu GC-MS-QP-2010 spectrometer. The molecular ion peak and the base peak, in all compounds, were clearly obtained in mass spectral study. The molecular ion peaks were found to be in agreement with molecular weight of the respective compounds. NMR Spectrum of compound $BLT_2$, was taken which showed methylene ($-CH_2$), amine (>NH), hydroxy (R-OH) methyl ($-CH_3$) and aromatic protons (Ar-H) peaks. The values for methylene ($-CH_2$) proton was observed between 2.77 and 2.86 δ ppm. Aromatic protons showed the multiplet between 7.35-8.05 δ ppm. The singlet peak of amine (-N<u>H</u>) was observed at 3.88-3.91 δ ppm. The value of methyl proton ($-C\underline{H}_3$) was observed as singlet at 1.18 δ ppm. Multiplet were observed at 4.26-4.29 ppm of ($-CH_2C\underline{H}CH_2$) proton and triplet was obtained at 4.16-4.22 ppm due to ($-CH_2C\underline{H}$) group.

For screening of anticoagulant activity, at the end of three weeks treatment, blood samples were collected from retro orbital plexuses under light ether anesthesia. The samples were collected in EDTA tube to prevent clot formation, at room temperature. Determination of clotting time was done, using Lee and white method

From each coumarin derivative, 4 compounds were selected for screening. Our aim was to evaluate anticoagulant property followed by evaluation of antihypertensive activity. Warfarin-treated (0.1mg/kg p.o.) rats showed significant increase in bleeding and clotting time, as compared to normal control rats. Treatment with, all 12 test compounds (5ml/kg/day, p.o) also

produced significant increase in bleeding and clotting time, as compared to normal control rats.

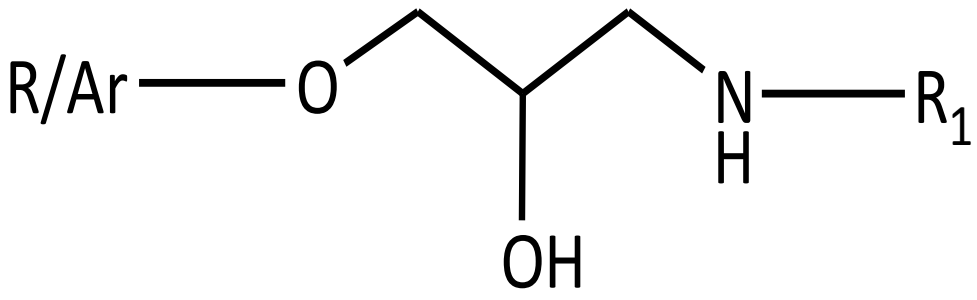
When subjected to pharmacological screening for anticoagulant activity and antihypertensive activity; all 12 synthesized compounds exhibited anticoagulant activity but highly significant anticoagulant activity was observed in compounds BLT $_{2, 8, 12, 18, 28}$ accompanied by increased bleeding and clotting time that was near to the standard drug viz. Warfarin, indicating that 7-methyl and 8-methyl derivatives also possess anticoagulant activity. Substituted groups that contain four carbon atoms; if substituted at nitrogen atom, exhibit maximum activity. This goes to confirm that 4-hydroxycoumarin derivatives, have anticoagulant property and that there is no relation between substituted and unsubstituted coumarin derivatives. We believe that in coumarin class anticoagulant compounds, hydroxyl group of 4-Hydroxy coumarin plays important role in formation of cyclic structure through hydrogen bond, with side chain attached to 3$^{rd}$ carbon in coumarin nucleus e.g. Warfarin. In case of our compounds, hydrogen bond formation takes place between hydroxyl group of side chain and pi-electrons of alkenes in coumarin ring (3$^{rd}$ and 4$^{th}$ carbons) hence; cyclic structure is formed showing activity in all compounds that were tested.

We believe that bulkier substitution on 3$^{rd}$ position of coumarin causes increase in anticoagulant activity, e.g. ferulenol. In our project, we found that more bulky groups like *tert*-butyl (BLT $_{1}$) showed significant anticoagulant activity. Diethanol derived compounds BLT $_{8}$ and BLT $_{18}$, showed good activity for the same reason. Finally, we conclude that formation of ring structure in coumarin through hydrogen bonding and bulky groups at terminal part of the side chain are responsible for anticoagulant activity, in a compound.

9 anticoagulant compounds were subjected to antihypertensive screening. Here, noradrenalin-treated (10mg/kg i. v.) rats showed significant increase in blood pressure. Treatment with test compounds (5mg/kg/day, i. v.) also produced antihypertensive action, but BLT $_{1, 2, 21}$ had significant effect as compared to others.

Compounds BLT $_{1, 2, 7, 11, 12, 17, 21, 22, 27}$ were evaluated for antihypertensive activity, using invasive method, on BioPac instrument. Results showed that Isopropyl and *tert*-butyl substituted compounds i.e. BLT $_{1, 2, 21}$ possess antihypertensive activity. While other compounds like BLT $_{7, 11, 12, 17, 22, 27}$ did not show significant antihypertensive activity. We infer that increase in no. of carbon atoms, more than four, on amine substitution, is likely to cause decrease in antihypertensive activity.

Thus, in the present project, we synthesized 30 coumarin derivative compounds, out of which, 12 compounds exhibited anticoagulant activity, 9 among which also exhibited antihypertensive activity. Further, some of these 8 compounds exhibiting dual activity were 7-methyl substituted coumarins. Literature review reveals that carbon no. 7 in coumarin nucleus of Warfarin molecule is involved in its metabolism, before being excreted in urine. Hence, metabolism studies of these compounds may be planned as an extension of our work.




# INTRODUCTION

# 2. INTRODUCTION

Cardiovascular diseases include a wide range of heart abnormalities, as well as diseases of other parts of the circulatory system, such as the coronary arteries, the cerebrovascular system, the aorta and pulmonary vessels, and the peripheral arteries and veins.[1] Cardiovascular disease is ravaging India and China. Unfortunately, though, the people of such low- and middle-income countries (LMIC) are not able to access preventive treatments widely available in the West. Cardiovascular disease was the leading cause of death globally in 2005 with more than 80 per cent of these deaths occurring in LMIC.[2] In China, stroke, chronic obstructive airways diseases, cancer and heart disease are the four highest contributors to the country's total disease burden almost half of these are due to cardiovascular disease. Cardiovascular disease risk factors such as obesity, high blood pressure, tobacco smoking and diabetes, are on the increase in LMIC. India, as an example, has twice the mortality rate from cardiovascular-related deaths among people of working age between 39 and 59 years, compared to the USA. Most patients in low- and middle-income countries have a choice between foregoing expensive treatment and taking financial ruin.[3] There is no health system in place to deliver the affordable drugs that can treat and prevent the disease burden for those in need cardiovascular drugs include antihypertensives, antihyperlipidemics, vasodilators, anticoagulants, diuretics and other Agents.

## 2.1 HYPERTENSION

Hypertension is the most common cardiovascular disease and a silent epidemic of the 20$^{th}$ century. According to the National Health Examination Survey of 1960 to 1962, 15 percent of whites and 27 percent of blacks had hypertension based on the World Health Organization criteria (blood pressure above 140/90 mm of mercury). Despite availability of adequate treatment, most hypertensive persons have inadequately controlled blood pressure. Both a lack of awareness and lack of compliance contribute to the problems.[4] The mortality associated with hypertension and hypertension-related myocardial infarctions, cerebrovascular accidents and renal failure account for more

than a million deaths per year in the United States. In addition, the morbidity associated with these diseases has been estimated to cost more than $5 billion a year.[5]

The third National Health and Nutrition Examination Survey (NHANES III), conducted from 1992 to 1994, found that 27% of the USA adult population had hypertension.[6] Essential hypertension cannot be cured but can be controlled. Many individuals experience hypertension as they grow older, but hypertension is not a part of healthy aging. For many older individuals, the systolic pressure gives the most accurate diagnosis of hypertension. Geographical variations in cardiovascular diseases and associated risk factors have been recognized worldwide. Little attention has been directed to potential differences in hypertension between Europe and North America. Hypertension is more prevalent in Europe than United state and Canada. Average B.P. was 136/83mmHg in the European countries while in United States it is 127/77 mmHg.[7]

According to World Health Report 2002, cardiovascular diseases (CVDs) will be the largest cause of death and disability by 2020 in India (WHO 2004). Reviews of studies on hypertension epidemiology in India have shown high prevalence in both urban and rural areas. Indian urban population studies from the mid 1950s to late 1990s used the older WHO guidelines for diagnosis (known hypertension or BP >160 mm Hg systolic and/or > 95 mm Hg diastolic). A significantly increasing adult prevalence of hypertension has been reported changing from 4.4% in Agra (1961), 6.4% in Rohtak (1975), 15.5% in Bombay (1980), 14.1% in Ludhiana (1985), 11.0% in Jaipur (1995), 11.6% in Delhi (1997), and 13.1% in Chandigarh (1999). Although there is a lower prevalence of hypertension in rural Indian populations, there has been a steady increase over time here as well. Reddy *et al.* also reported high prevalence of hypertension in a study among industrial populations at multiple sites in India. These findings are in consonance with many developed countries where it has been reported that at any given time almost half of all individuals have high BP.[8]

The physiologic basis for essential hypertension is elevated peripheral resistance. Cardiac output is usually within normal limits. Only in a small minority of hypertensive patients is increased cardiac output the cause of hypertension. This subgroup of hypertensive patients tends to be younger and has a more labile pattern of blood pressure elevations.[9-10]

The cause of most forms of hypertension is unfortunately not known. There is a likelihood that essential hypertension has multiple causes, although evidence to support this is lacking. Measurements of renin activity in hypertensive patients has received considerable attention recently, but categorizing patients by their renin levels has not improved blood pressure control and, at present, merely adds to the already skyrocketing cost of medical care.[11-16] Hypertension damages blood vessels in kidney, heart, and brain and leads to an increased incidence of renal failure, coronary disease, cardiac failure, and stroke. Hypertension is a major risk factor for two distinct kinds of vascular problems: Complications of atherosclerosis including myocardial and cerebral infraction as well as complications of small vessel disease including renal failure, intracerebral hemorrhage and lacunar infractions.[17] As a consequence of elevated blood pressure, arterial elasticity is reduced and wall damage appears which can lead to cholesterol and fat deposition on those lesions and eventually to obstruction of the vessels. This is the basis of most of the target organ damages induced by hypertension. At very high blood pressures (systolic 210 and/or diastolic 120 mm Hg), a subset of patients develops fulminant arteriopathy characterized by endothelial injury and a marked proliferation of cells in the intima, leading to intimal thickening and ultimately to arteriolar occlusion. This is the pathological basis of the syndrome of immediately life-threatening hypertension, which is associated with rapidly progressive microvascular occlusive disease in the kidney (with renal failure), brain (hypertensive encephalopathy), congestive heart failure, and pulmonary edema.

Another consequence can be the increase in vascular resistance, which forces the pumping activity of the heart to maintain its role in nutrients and oxygen distribution. This work overload for the heart may induce the development of cardiac hypertrophy,

an increase in cardiac mass and thickness. Left ventricular hypertrophy defined by electrocardiogram, or more sensitively by echocardiography, is associated with a substantially worse long-term outcome that includes a higher risk of sudden cardiac death. The risk of cardiovascular disease, disability, and death in hypertensive patients also is increased markedly by concomitant cigarette smoking, diabetes, or elevated low-density lipoprotein; the coexistence of hypertension with these risk factors increases cardiovascular morbidity and mortality to a degree that is compounded by each additional risk factor. Since the purpose of treating hypertension is to decrease cardiovascular risk, other dietary and pharmacological interventions may be required.[18]

### 2.1.1 Antihypertensive Agents

Therapy using the antihypertensive agents evolved rapidly between 1950 and 1960. During that time a number of empiric discoveries were made that resulted in the marketing of the drugs for the treatment and control of hypertensive diseases. Nonpharmacological therapy is an important component of treatment of all patients with hypertension. In some stage 1 hypertension, blood pressure may be adequately controlled by a combination of weight loss (in overweight individuals), restricting sodium intake, increasing aerobic exercise, and moderating consumption of alcohol. These lifestyle changes, though difficult for many to implement, may facilitate pharmacological control of blood pressure in patients whose responses to lifestyle changes alone are insufficient.

The conventional treatment for the hypertension includes various drugs from the categories of diuretics, β-blockers, calcium channel blockers, ACE Inhibitors, Type-1 angiotensin II receptor antagonists, $\alpha_1$ -adrenoceptor antagonists, vasodilators etc.

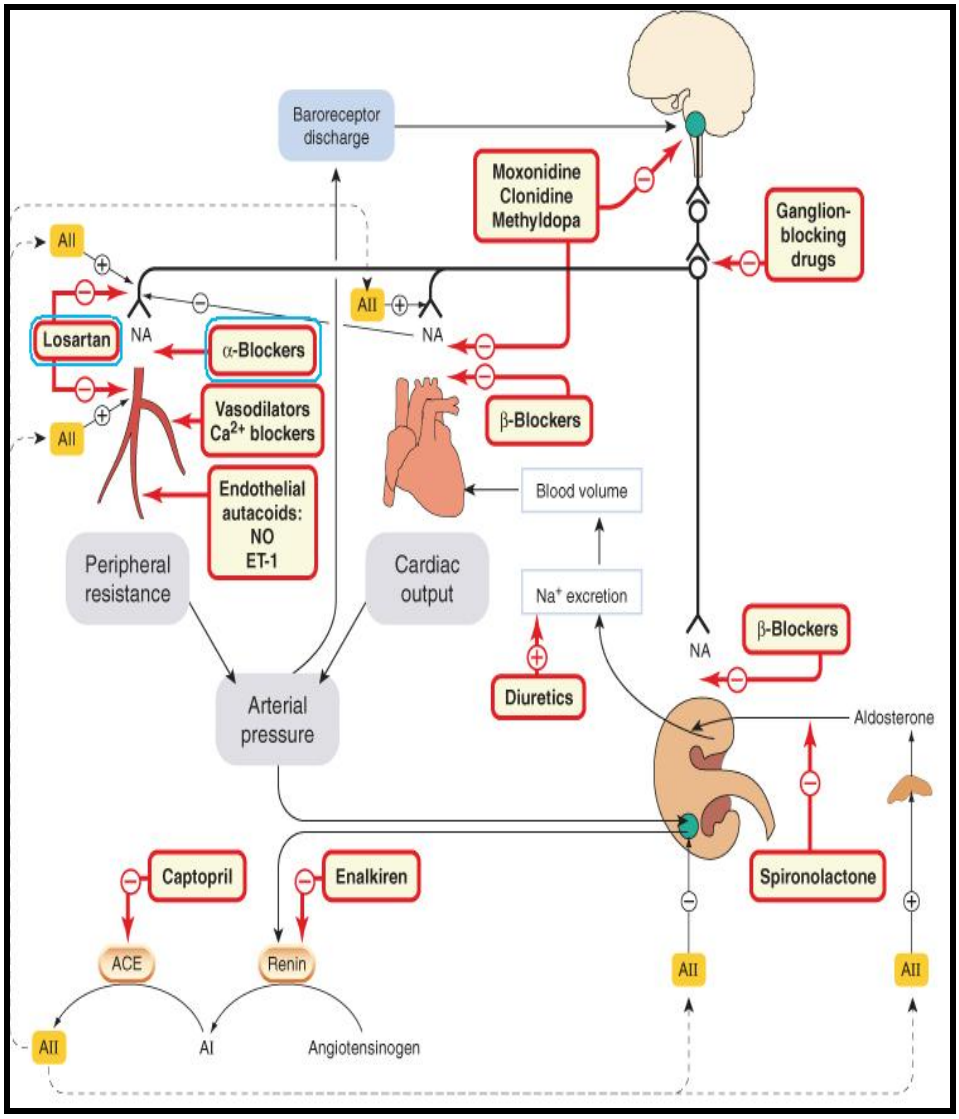



**Fig. 1 Target for Antihypertensive Treatment**[19]

**Table- 1 Various Classes of Drugs for the Treatment of Hypertension**[19]

| Class of drugs | Mode of action | Adverse reactions |
|---|---|---|
| β-adrenoceptor antagonists (propranolol, atenolol, metoprolol) | Receptor blockade– decreased cardiac output and peripheral vascular resistance | Bronchoconstriction, fatigue, depression, nightmares |
| ACE Inhibitors (captopril, lisinopril, enalapril) | Inhibits angiotensin converting enzyme– reduces the formation of Ang-II | Chronic cough, hyperkalemia, first dose hypotension, renal failure |
| Centrally acting drugs (clonidine, methyl dopa) | Stimulates $\alpha_2$-adrenoceptor –inhibits | Dryness of mouth, drowsiness, depression, |

| | | |
|---|---|---|
| | release of norepinephrine | rebound hypertension |
| Diuretics (hydrochlorothiazide frusemide, spironolactone) | Sodium excretion, volume depletion | Urinary frequency, gout, Glucose intolerance, hypokalemia, thrombocytopenia |
| Calcium channel blockers (verapamil, diltiazem, amlodipine, nifedipine) | Relaxes vascular smooth muscles–decreases Peripheral vascular resistance | Edema, dizziness, nausea, palpitation |
| $\alpha_1$-Adrenoceptor antagonists (prazosin,terazosin,doxazosin) | $\alpha_1$-Adrenoceptor blockade–vasodilatation | Postural hypotension, Paroxysmal tachycardia, nightmares |
| Angiotensin II receptor antagonists (losartan,Valsartan) | Block angiotensin-II receptor on the arteries | hyperkalemia, first dose hypotension |

Arterial pressure is the product of cardiac output and peripheral vascular resistance. Drugs lower blood pressure by actions on peripheral resistance, cardiac output, or both. Drugs may reduce the cardiac output by inhibiting myocardial contractility or by decreasing ventricular filling pressure. Reduction in ventricular filling pressure may be achieved by actions on the venous tone or on blood volume *via* renal effects. Drugs can reduce peripheral resistance by acting on smooth muscle to cause relaxation of resistance vessels or by interfering with the activity of systems that produce constriction of resistance vessels (*e.g.,* the sympathetic nervous system).

In patients with isolated systolic hypertension, complex hemodynamics in a rigid arterial system contribute to increased blood pressure; drug effects may be mediated by changes in peripheral resistance but also *via* effects on large artery stiffness The hemodynamic consequences of long-term treatment with antihypertensive agents provide a rationale for potential complementary effects of concurrent therapy with two or more drugs. The simultaneous use of drugs with similar mechanisms of action and hemodynamic effects often produces little additional benefit. However, concurrent use of drugs from different classes is a strategy for achieving effective control of blood pressure while minimizing dose-related adverse effects.

It is generally not possible to predict the responses of individuals with hypertension to any specific drug. For example, for some antihypertensive drugs, on average about two-thirds of patients will have a meaningful clinical response, whereas about one-third of patients will not respond to the same drug. There is considerable interest in identifying genetic variation in order to improve selection of antihypertensive drugs in individual patients. Polymorphisms in a number of genes involved in the metabolism of antihypertensive drugs have been identified, for example in the CYP family (phase I metabolism) and in phase II metabolism, such as catechol-O-methyltransferase. While these polymorphisms change the pharmacokinetics of specific drugs, it is not clear that there will be substantial differences in efficacy given the dose range available clinically for these drugs.

One of the biggest problems in the treatment of hypertension is patient compliance. It would seem logical that improving compliance would improve the overall treatment of hypertension. Because all drugs have side effects, it is important to individually tailor therapy to minimize discomfort to each patient. However, the patient must take responsibility for his illness and realize that his life-style may have to be altered slightly if he is to live longer. One basic principle that should apply to all drug therapy is that the less frequently a drug is taken, the more likely it is that the drug will be taken. Thus, a once-a-day regimen is better than twice daily, but twice a day is better than four times a day. Because almost all antihypertensive drugs can be given twice per day, compliance from this point of view should be good. Also, the fewer the drugs used to control blood pressure, the easier will be compliance. If blood pressure could be controlled using one drug once a day, overall compliance would be expected to improve.

Another major problem in treating hypertensive patients is that most of them are asymptomatic; however, annoying side effects develop after initiation of drug therapy. Unless patients are well educated about the consequences of hypertension, it is easy to understand why many do not continue to take their medication. Implementing some self-assessment of blood pressure control has been shown to be helpful in overall

compliance.[20] If a patient were to record his own blood pressure two times a day, not only would the physician get a better idea of his patient's hypertensive control, but the patient also would have some concrete goals to work towards that he could assess each day. This form of self-assessment of control would be similar to that practiced by diabetic patients who check urine glucose levels.

Recommendations for specific drugs in treating hypertension are difficult to make unless the overall life-style and habits of each patient are evaluated individually. Obviously, if a patient makes his living as a bus driver, drugs like methyldopa and clonidine will be inappropriate because of central depressive effects. A patient who is athletically active may not do well taking high doses of propranolol because of the drug's ability to inhibit exercise-induced tachycardia. However, this same patient may find low doses of propranolol combined with a peripheral vasodilator to be a satisfactory regimen. In a patient with adult-onset diabetes this condition may be made worse by thiazide diuretics because the drugs interfere with insulin release. Yet, propranolol may be effective in such a patient. On the other hand, an insulin-dependent diabetic patient may find the use of propranolol unacceptable because it interferes with the warning signs and symptoms of hypoglycemia. Ideally, an antihypertensive regimen should be simple (one or two drugs, twice a day) and each patient should be made aware of possible side effects of the drugs before beginning therapy. Patients should actively participate in monitoring the control of their illness. And physicians should work with patients so that together they can control blood pressure with the fewest drug side effects.

### 2.1.2 β-Blockers

#### 2.1.2.1 Pharmacology

Antagonism of β-adrenergic receptors affects the regulation of the circulation through a number of mechanisms, including a reduction in myocardial contractility, heart rate, and cardiac output. An important consequence of using β - adrenergic receptors is blockade of the β receptors of the juxtaglomerular complex, reducing renin secretion and thereby diminishing production of circulating angiotensin II. This action likely

contributes to the antihypertensive action of this class of drugs, in concert with the cardiac effects.

The β-adrenergic blockers vary in their lipid solubility, selectivity for the $\beta_1$-adrenergic receptor subtype, presence of partial agonist or intrinsic sympathomimetic activity, and membrane-stabilizing properties. Despite these differences, all of the β adrenergic receptor antagonists are effective as antihypertensive agents. However, these differences do influence the clinical pharmacokinetics and spectrum of adverse effects of the various drugs. Drugs without intrinsic sympathomimetic activity produce an initial reduction in cardiac output and a reflex-induced rise in peripheral resistance, generally with no net change in arterial pressure. In patients who respond with a reduction in blood pressure, peripheral resistance gradually returns to pretreatment values or less. Generally, persistently reduced cardiac output and possibly decreased peripheral resistance accounts for the reduction in arterial pressure. Drugs with intrinsic sympathomimetic activity produce lesser decreases in resting heart rate and cardiac output; the fall in arterial pressure correlates with a fall in vascular resistance below pretreatment levels, possibly because of stimulation of vascular $\alpha_2$ adrenergic receptors that mediate vasodilation. Drugs without intrinsic sympathomimetic activity produce an initial reduction in cardiac output and a reflex-induced rise in peripheral resistance, generally with no net change in arterial pressure. In patients who respond with a reduction in blood pressure, peripheral resistance gradually returns to pretreatment values or less. Generally, persistently reduced cardiac output and possibly decreased peripheral resistance accounts for the reduction in arterial pressure. Drugs with intrinsic sympathomimetic activity produce lesser decreases in resting heart rate and cardiac output; the fall in arterial pressure correlates with a fall in vascular resistance below pretreatment levels, possibly because of stimulation of vascular $\beta_2$ adrenergic receptors that mediate vasodilatation.

Propranolol is the proto type agent for class of β-adregernic blocking agents. Propranolol has equal affinity for $\beta_1$ and $\beta_2$ adrenergic receptors; thus, it is a nonselective β adrenergic receptor antagonist. Propranolol is a pure antagonist, lacks intrinsic sympathomimetic activity. For the treatment of hypertension and angina, the

initial oral dose of propranolol generally is 40 to 80 mg per day. The dose may then be titrated upward until the optimal response is obtained. For the treatment of angina, the dose may be increased at intervals of less than 1 week, as indicated clinically. Propranolol is also used to treat supraventricular arrhythmias/tachycardias, ventricular arrhythmias/tachycardias, premature ventricular contractions, digitalis-induced tachyarrhythmias, myocardial infarction, pheochromocytoma, and the prophylaxis of migraine. It also has been used for several off-label indications including parkinsonian tremors (sustained-release only), akathisia induced by antipsychotic drugs, variceal bleeding in portal hypertension, and generalized anxiety disorder. Propranolol may be administered intravenously for the management of life-threatening arrhythmias or to patients under anesthesia.[21] Because all β adrenergic receptor antagonists are effective antihypertensive agents and (+)-propranolol, the inactive isomer that has little β-adrenergic receptor blocking activity, has no effect on blood pressure, the antihypertensive therapeutic effect of these agents is undoubtedly related to receptor blockade.[22]

Propranolol is highly lipophilic and is almost completely absorbed after oral administration. However, much of the drug is metabolized by the liver during its first passage through the portal circulation; on average, only about 25% reaches the systemic circulation. In addition, there is great inter individual variation in the presystemic clearance of propranolol by the liver; this contributes to enormous variability in plasma concentrations (approximately twentyfold) after oral administration of the drug and contributes to the wide range of doses in terms of clinical efficacy. A clinical disadvantage of propranolol is that multiple, increasing steps in drug dose may be required over time. The degree of hepatic extraction of propranolol declines as the dose is increased. The bioavailability of propranolol may be increased by the concomitant ingestion of food and during long-term administration of the drug. Propranolol has a large volume of distribution (4 liters/kg) and readily enters the CNS. Approximately 90% of the drug in the circulation is bound to plasma proteins. It is extensively metabolized, with most metabolites appearing in the urine. One product of hepatic metabolism is 4-hydroxypropranolol, which has some β adrenergic antagonist activity.

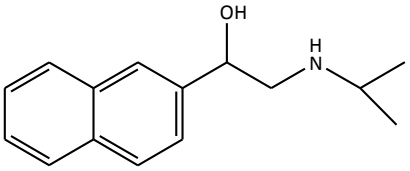
Analysis of the distribution of propranolol, its clearance by the liver, and its activity is complicated by the stereospecificity of these processes. The (-)-enantiomers of propranolol and other β blockers are the active forms of the drug. The (-)-enantiomer of propranolol appears to be cleared more slowly from the body than is the inactive enantiomer. The clearance of propranolol may vary with hepatic blood flow and liver disease and also may change during the administration of other drugs that affect hepatic metabolism. Monitoring of plasma concentrations of propranolol has found little application, since the clinical endpoints (reduction of blood pressure and heart rate) are readily determined. The relationships between the plasma concentrations of propranolol and its pharmacodynamic effects are complex; for example, despite its short half-life in plasma (about 4 hours), its antihypertensive effect is sufficiently long-lived to permit administration twice daily. Some of the (-)-enantiomer of propranolol and other β blockers is taken up into sympathetic nerve endings and is released upon sympathetic nerve stimulation.[23]

#### 2.1.2.2 Adverse Effects of β-blockers

B-blockers should be avoided in patients with reactive airway disease (asthma) or with sinoatrial or atrioventricular (AV) nodal dysfunction or in combination with other drugs that inhibit AV conduction, such as verapamil. Patients with insulin-dependent diabetes also are better treated with other drugs. β Receptor antagonists without intrinsic sympathomimetic activity increase concentrations of triglycerides in plasma and lower those of HDL cholesterol without changing total cholesterol concentrations. β -adrenergic blocking agents with intrinsic sympathomimetic activity have little or no effect on blood lipids or increase HDL cholesterol. The long-term consequences of these effects are unknown. Sudden discontinuation of some β adrenergic blockers can produce a withdrawal syndrome that is likely due to up-regulation of β receptors during blockade, causing enhanced tissue sensitivity to endogenous catecholamines; this can exacerbate the symptoms of coronary artery disease. The result, especially in active patients, can be rebound hypertension. Thus, β adrenergic blockers should not be discontinued abruptly except under close observation; dosage should be tapered over 10 to 14 days prior to discontinuation.

### 2.1.2.3 Chemistry of β - blockers

In medicinal chemistry, the chemist attempts to design and synthesize a medicine or a pharmaceutical agent which will benefit humanity. Recently, several clinical trials have demonstrated that beta-blockers remarkably reduced mortality in patients with moderate heart failure as well as improved the quality of life and sense of well-being by reducing hospitalizations and arrhythmias.[24] β - Blockers are all structurally similar agents known as aryloxypropanolamines. This name originates from the presences of an $–OCH_2-$ group located between a substituted aromatic ring and an ethylamino side chain of pronethalol (1). The aromatic ring and substituents are the primary determinants of β antagonist selectivity. Propranolol (2), nadolol (3), timolol (4) and pindolol (5) are non-specific β blockers whereas para position substitutions on the aromatic rings of metoprolol (6), atenolol (7), esmolol (8), acebutolol (9) both confer $\beta_1$ antagonist selectivity.[25]

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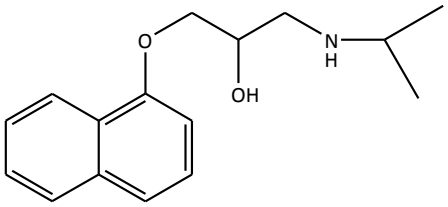


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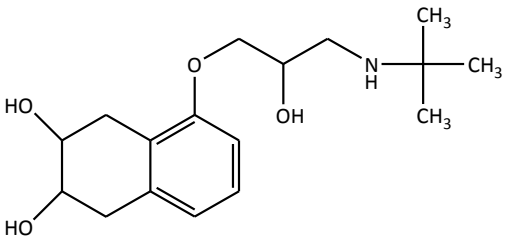


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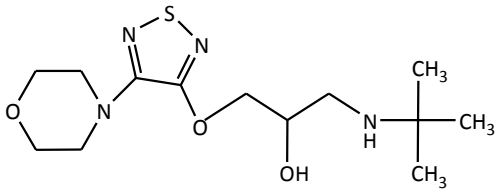


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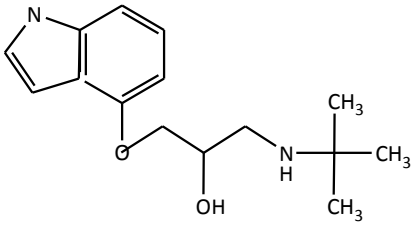

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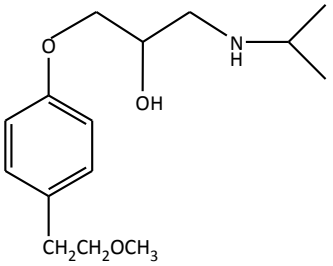

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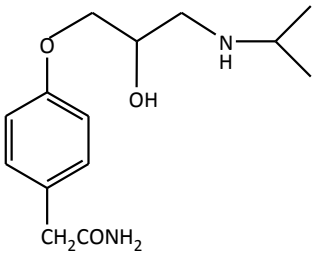

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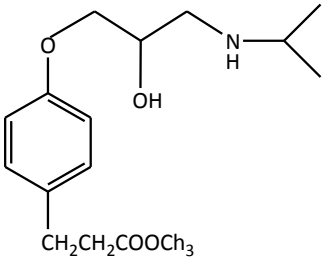

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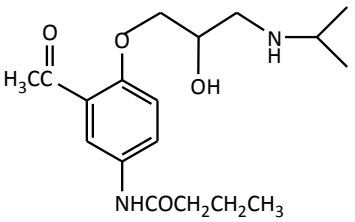

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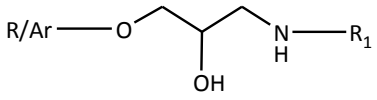
## 2.2 ALKYLAMINOHYDROXYPROPOXY SIDECHAIN DERIVATIVES

The concept of alkyl aminohydroxypropoxy (10) side chain derivatives is understood by class of β – adrenergic blocking agents. Researchers who were studying the effects of aryl ring and aryl substitution in the molecule tried to modify the ethanolamine chain itself by inter alia, the introduction of linking group between aryl ring and ethanolamine chain. After lots of linking groups tried, the best linking group comes out to be oxymethylene. It is first analog, propranolol which is the most widely used β - blocker now and is 10 to 20 times potent than its parent compound pronethalol.[26] Propranolol belongs to the group of β-blocking agents known as aryloxypropanolamines. Bulky aliphatic groups, such as the *tert*-butyl and isopropyl groups, are normally found on the amino function of the aryloxypropanolamine β-receptor antagonist. The configuration of the hydroxyl-bearing carbon of the aryloxypropanolamine side chain plays a critical role in the interaction of β-blocking agents with β receptors. The aryl group affect on pharmacokinetic parameter of the β-blockers.

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## 2.3 THROMBO-EMBOLISM

Thrombosis is the formation of a blood clot (thrombus) inside a blood vessel, obstructing the flow of blood through the circulatory system. When a blood vessel is injured, the body uses platelets and fibrin to form a blood clot to prevent blood loss. If the clotting is too severe and the clot breaks free, the traveling clot is now known as an embolus. Sometimes, part, or all, of a blood clot can come away from its original site and travel through the bloodstream. If this occurs, the clot can become lodged in another part of the body. This is known as an embolism. Thromboembolism is the combination of thrombosis and its main complication, embolism. When a thrombus occupies more than 75% of surface area of the lumen of an artery, blood flow to the tissue supplied is reduced enough to cause symptoms because of decreased oxygen (hypoxia) and accumulation of metabolic products like lactic acid. More than 90% obstruction can result in anoxia, the complete deprivation of oxygen, and infarction, a mode of cell death. The symptoms of a thromboembolism depend on the organ or blood vessel that has lost blood supply. Blood clots in an arm or leg may cause pain, swelling, and increased temperature in the affected area. A clot that travels to the lung is called a pulmonary embolus. This condition can cause: chest pain, shortness of breath, rapid heartbeat, known as tachycardia, fainting or death. If a blood clot is formed in the heart, it can travel to almost any organ in the body. This could cause a stroke, which is a type of damage to the brain from lack of blood circulation. In other cases, damage may be done to an arm or leg, or a heart attack or kidney damage may occur. Other areas of the body can also be affected.

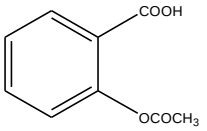
### 2.3.1 Antithrombic Treatment

An antithrombotic is a drug which reduces thrombus formation. Different antithrombotic affect different processes:

1. Antiplatelet drugs - limit the migration or aggregation of platelets.
2. Thrombolytic drugs - act to dissolve clots after they have formed.
3. Anticoagulants - limit the ability of the blood to clot.

### 2.3.1.1 Antiplatelet Drugs

An Antiplatelet drug (antiaggregant) is a member of a class of pharmaceuticals that decrease platelet aggregation[27] and inhibit thrombus formation. The most important antiplatelet drugs are: (a) Cyclooxygenase inhibitors e.g. Aspirin (11) (b) Adenosine diphosphate (ADP) receptor inhibitors e.g. Clopidogrel (12), Prasugrel (13), Ticlopidine (Ticlid) (14) (c) Phosphodiesterase inhibitors e.g. Cilostazol (15) (d) Glycoprotein IIB/IIIA inhibitors e.g. Abciximab.

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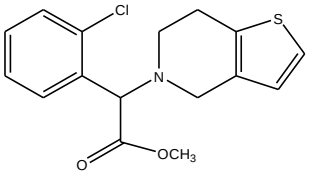


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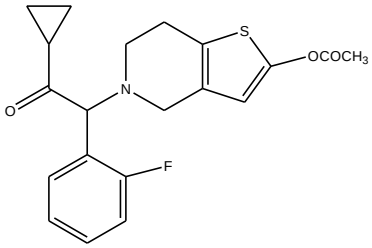


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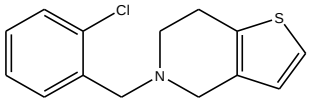


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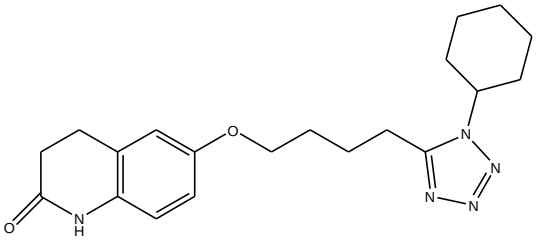


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### 2.3.1.2 Thrombolytic Drugs

**Thrombolytic drugs** are used to dissolve blood clots in a procedure termed thrombolysis. They limit the damage caused by the blockage of the blood vessel. Thrombolysis is used in myocardial infarction (heart attack), thromboembolic strokes, deep vein thrombosis and pulmonary embolism to clear a blocked artery and avoid permanent damage to

the perfused tissue. The thrombolytic drugs include: a) tissue plasminogen activator e.g. alteplase, reteplase, anisteplase, streptokinase, urokinase.

### 2.3.1.3 Anticoagulants

An anticoagulant is a substance that prevents coagulation; that is, it stops blood from clotting. Anticoagulants were introduced into medical practice more than three decades ago. Extensive use of these drugs in the prevention and treatment of thromboembolic disease has made them one of the most widely used classes of pharmacological agents. Anticoagulant drugs include:

a) Heparin and derivative substances e.g. Low molecular weight heparin

b) Vitamin K antagonists e.g. Warfarin

#### 2.3.1.3.1 Warfarin

Warfarin and related 4-hydroxycoumarin-containing molecules decrease blood coagulation by inhibiting vitamin K epoxide reductase, an enzyme that recycles oxidized vitamin K to its reduced form after it has participated in the carboxylation of several blood coagulation proteins, mainly prothrombin and factor VII. For this reason, drugs in this class are also referred to as vitamin K antagonists.[2] When administered, these drugs do not anticoagulate blood immediately. Instead, onset of their effect requires about a day before clotting factors being normally made by the liver have time to naturally disappear in metabolism, and the duration of action of a single dose of racemic warfarin is 2 to 5 days. Under normal pharmacological therapy the drugs are administered to decrease the action of the clotting factors they affect by 30 to 50%.[3]

Therapeutic doses of warfarin decrease by 30% to 50% the total amount of each vitamin K-dependent coagulation factor made by the liver; in addition, the secreted molecules are undercarboxylated, resulting in diminished biological activity (10% to 40% of normal). Congenital deficiencies of the procoagulant proteins to these levels cause mild bleeding disorders. Oral anticoagulants have no effect on the activity of fully carboxylated molecules in the circulation. Thus, the time required for the activity of each factor in plasma to reach a new steady state after therapy is initiated or adjusted depends on its individual rate of clearance. The approximate half-lives (in hours) are as

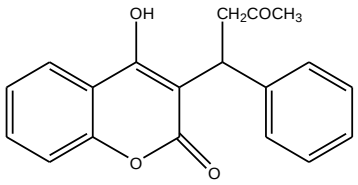
follows: factor VII, 6; factor IX, 24; factor X, 36; factor II, 50; protein C, 8; and protein S, 30. Because of the long half-lives of some of the coagulation factors, in particular factor II, the full antithrombotic effect of warfarin is not achieved for several days, even though the PT may be prolonged soon after administration due to the more rapid reduction of factors with a shorter half-life, in particular factor VII. There is no obvious selectivity of the effect of warfarin on any particular vitamin K-dependent coagulation factor, although the antithrombotic benefit and the hemorrhagic risk of therapy may be correlated with the functional level of prothrombin, and to a lesser extent, factor X.[28]

The usual adult dose of warfarin is 5 mg per day for 2 to 4 days, followed by 2 to 10 mg per day as indicated by measurements of the international normalized ratio (INR), a value derived from the patient's PT (*see* functional definition of INR in section on laboratory monitoring, below). A lower initial dose should be given to patients with an increased risk of bleeding, including the elderly. Warfarin usually is administered orally; age correlates with increased sensitivity to oral anticoagulants. Warfarin also can be given intravenously without modification of the dose. Intramuscular injection is not recommended because of the risk of hematoma formation.

Warfarin is a synthetic derivative of dicoumarol, a 4-hydroxycoumarin-derived mycotoxin anticoagulant originally discovered in spoiled sweet clover-based animal feeds. Dicoumarol, in turn, is derived from coumarin, a sweet-smelling but coagulation-inactive chemical found naturally in "sweet" clover (to which it gives its odor and name) and many other plants. The name *warfarin* stems from its discovery at the University of Wisconsin, incorporating the acronym for the organization which funded the key research (*WARF*, for Wisconsin Alumni Research Foundation) and the ending -arin, indicating its link with coumarin.

## 2.4 COUMARIN

The story of the coumarin anticoagulants generally is traced back to the early 1920s, when the "sweet clover disease" showed up almost simultaneously in North Dakota and in Alberta, Canada. This new malady of cattle involving fatal bleeding was traced to stacks of sweet clover hay.[29] Professor Link and his students at the Wisconsin Agriculture Experiment Station set out to identify the hemorrhagic agent in 1934 and first struggled with developing a reliable bioassay. After mass isolation of the active ingredients, its structure was diagnosed as 3, 3'- methylenebis-(4-hydroxycoumarin) (17). Thousands of synthetic equivalents were prepared in 1940. Since 1940, hundreds of 4-hydroxycoumarin-based compounds have been prepared and tested for anticoagulant activity, among these; warfarin quickly became the leader in clinical anticoagulant therapy.

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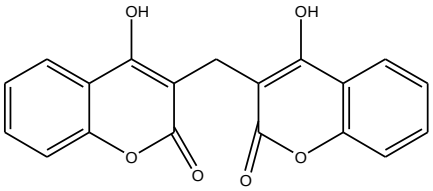


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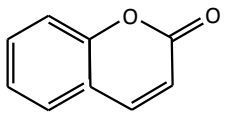
Although the coumarin anticoagulants have prolonged the lives of many patients during the first three decades of their therapeutic application (simultaneously promoting human welfare via pest control), our understanding of many basic aspects of these drugs is still meager. Only in recent years, detailed information on the metabolic fate of one of these compounds (warfarin) becomes known. No information has been presented for the other drugs of this group –not even for the analogs, although such studies would seem to be greatly simplified by following a protocol parallel to the warfarin work**.** Formerly, large quantities of coumarin were used in the food industry, mostly associated with vanillin, for flavouring chocolates, baked goods, and in cream soda-flavored beverages, but since 1954 its use as a direct food additive has been suspended in the United States.[30]

### 2.4.1 Chemistry of Coumarin

Structural formula:



Empirical formula: $C_9H_6O_2$

Relative molecular mass: 146.15

**Nomenclature of coumarin**

Chem. Abstract Name: 2H-1-Benzopyran-2-one

IUPAC Systematic Name: Coumarin

Synonyms: 1,2-Benzopyrone

**Physical and chemical properties**

(a) Description: Orthorhombic, rectangular plates; pleasant, fragrant odor resembling that of vanilla beans; burning taste

(b) Boiling-point: 301.7 °C

(c) Melting-point: 71 °C

(d) Density: 0.935 g/cm$^3$ at 20 °C

(e) Spectroscopy: Infrared (prism [1691]; grating [270]), ultraviolet [492], nuclear magnetic resonance (proton, [10407, V-225]; C-13 [242]) and mass spectral data have been reported

(f) Solubility: Slightly soluble in water (100 mg/L at 25 °C) and ethanol; very soluble in chloroform, diethyl ether and pyridine

(g) Volatility: Vapour pressure, 0.13 kPa at 106 °C

(h) Octanol/water partition coefficient (P): log P, 1.39 (i) Conversion factor 1: mg/m$^3$ = 5.98 × ppm

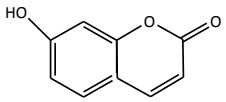
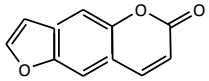
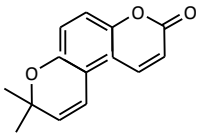
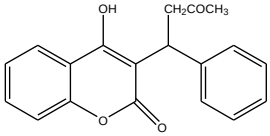
Coumarin has a characteristic hay-like, sweet aromatic creamy odor with certain nutty shadings, much used in synthetic form as a fragrance chemical for various products like toothpastes, antiperspirant deodorants, bath products, body lotions, face creams, fragrance creams, hair sprays, shampoos, shower gels and toilet soaps. Coumarin has been used in detergents as a brightener or bleaching agent. It is used as an odor-enhancer to achieve a long-lasting effect when combined with natural essential oils such as lavender, citrus, rosemary and oak moss. Coumarin is used in tobacco to enhance its natural aroma. It is also applied in large quantities to give pleasant aromas to house- hold materials and industrial products or to mask unpleasant odours.

Coumarin is a widely occurring secondary metabolite that occurs naturally in several plant families and essential oils. Coumarins, the name of this chemical family is derived from *Coumarouna odorata Aube (Dipteryx odorata) tonka beans* , from which was isolated, for the first time, the simplest member of this class, coumarin itself. A broad spectrum of coumarin derivatives (present both in the free state and as glucosides) have also been found in many plants; to date at least 1300 have been identified, principally as secondary metabolites in green plants (Hoult 1996). Until the late 1890s, coumarin was obtained commercially only from natural sources by extraction from tonka beans. Synthetic methods of preparation and industrial manufacturing processes were developed starting principally from *ortho*-cresol (Raschig process), phenol (Pechmann reaction) and salicylaldehyde (Perkin reaction). Various methods can be used to obtain coumarin from each of these starting materials. In order to be suitable for perfumery uses, synthetic coumarin must be highly pure. Information available in 1999 indicated that coumarin was manufactured by five companies in China, three companies each in Japan and the United States, two companies in France and one company each in Germany, Hong Kong and India. Coumarin is commercially available with a minimum purity of 99%.[31] Coumarin is usually sold in the form of colorless shiny leaflets or rhombic crystals.

Coumarin is an anhydride of o-coumaric acid (2H-1-benzopyran-2-one) having white, crystalline lactone, obtainable naturally from several plants, such as tonka bean,

lavender, sweet clover grass, strawberries, and cinnamon, or produced synthetically from an amino acid, phenylalanine. Bio synthetically, coumarin nucleus (benzo-2-pyrone) is derived from cinnamicacid (phenylacrylic skeleton). Accordingly, 7$^{th}$ position of the hydroxy group attached to coumarin structure is important in biosynthesis pathway, i.e. Umbelliferone (7-hydroxy coumarin), esculetin (6,7-Dihydroxycoumarin), scopoletin (7-hydroxy-6-methoxycoumarin) are the widespread coumarins in nature.

**Table 2: Types and Examples of Coumarins** [32]

| Classification | Features | Examples |
|---|---|---|
| Simple coumarins | Hydroxylated, Alkylated, Alkoxylated on benzene ring | 7- Hydroxy Coumarin  |
| Furano coumarins | 5 membered furan ring attached on benzene ring | Psoralen  |
| Pyranocoumarins | Six membered pyrone ring attached on benzene ring | Seselin  |
| Pyrone Substituted coumarines | Substitution on pyrone ring | Warfarin  |

Warfarin was synthesized by Ikawa in 1942 in the laboratory of Professor Link and the discovery of its high anticoagulant activity in rats, found extensive application as a rodenticide. Warfarin was introduced into clinical anticoagulant therapy in 1952. Since 195S, sodium warfarin has been the leading anticoagulant in clinical practice. It is estimated that about 85% of the oral anticoagulation in the United States is via sodiumwarfarin (Coumadin). Following the report of a hemorrhagic disorder in cattle that resulted from the ingestion of spoiled sweet clover silage, Campbell and Link, in 1939, identified the hemorrhagic agent as bishydroxycoumarin (dicoumarol). In 1948, a more potent synthetic congener was introduced as an extremely effective rodenticide; the compound was named *warfarin* as an acronym derived from the name of the patent holder, Wisconsin Alumni Research Foundation. Warfarin's potential as a therapeutic anticoagulant was recognized but not widely accepted, partly due to fear of unacceptable toxicity. However, in 1951, an Army inductee uneventfully survived an attempted suicide with massive doses of a preparation of warfarin intended for rodent control. Since then, these anticoagulants have become a mainstay for prevention of thromboembolic disease.

Numerous anticoagulants have been synthesized as derivatives of 4-hydroxycoumarin and of the related compound, indan-1,3-dione. Only the coumarin derivatives are widely used; the 4-hydroxycoumarin residue, with a nonpolar carbon substituent at the 3 position, is the minimal structural requirement for activity. This carbon is asymmetrical in warfarin (and in *phenprocoumon* and *acenocoumarol*). The enantiomers differ in anticoagulant potency, metabolism, elimination, and interactions with other drugs. Commercial preparations of these anticoagulants are racemic mixtures. No advantage of administering a single enantiomer has been established. The oral anticoagulants are antagonists of vitamin K (*see* section on vitamin K, below). Coagulation factors II, VII, IX, and X and the anticoagulant proteins C and S are synthesized mainly in the liver and are biologically inactive unless 9 to 13 of the amino-terminal glutamate residues are carboxylated to form the $Ca^{2+}$-binding g-carboxyglutamate (Gla) residues. This reaction of the descarboxy precursor protein requires carbon dioxide, molecular oxygen, and reduced vitamin K, and is catalyzed by g-glutamyl carboxylase in the rough endoplasmic reticulum. Carboxylation is directly

coupled to the oxidation of vitamin K to its corresponding epoxide.

Coumarin analogue, dicumarol[33] and its synthetic derivative warfarin sodium having an anticoagulant nature are used for the treatment of a variety of cancers. They act by disorganizing the mitotic spindle microtubules in cells, leading to the random distribution of the chromosomes at metaphase. Because of its low toxicity and simple chemical structure, there is potential interest to explore combinations random antimitotic coumarins with other chemotherapeutic agents to improve efficacy and lower toxicity of anti cancer drugs. Coumarin anticoagulants inhibit the pathway involving tissue factor and factor VIIa. Tissue factor VIIa appears to be a major factor in the regulation of angiogenic growth properties of tumor cells.[34]

Coumarin has no anticoagulant activity; it is transformed into the natural anticoagulant dicoumarol by a number of species of fungi. This occurs as the result of the production of 4-hydroxycoumarin, then further (in the presence of naturally occurring formaldehyde) into the actual anticoagulant dicoumarol, a fermentation product and mycotoxin. This substance was responsible for the bleeding disease known historically as "sweet clover disease" in cattle eating moldy sweet clover silage. Coumarin possesses immunomodulatory and direct antitumor activity. Coumarin has been recommended for treatment of a number of clinical conditions, including high protein oedema and brucellosis. It is currently undergoing clinical trials for treatment of lymphoedema following breast cancer treatment and in treatment of lung and kidney cancer and of melanoma alone or in combination with cimetidine. It has also been used for preven- tion of dental caries. Coumarin and some of its derivatives have been tested for treatment of schizophrenia, microcirculation disorders and angiopathic ulcers, and also for treatment of high protein oedemas in animals.

Coumarin derivatives are used as therapeutic anticoagulants and as rodenticides by causing fatal hemorrhage. Because the range between efficient therapy and undue hemorrhagic risk may vary greatly from one patient to another, the need for carefully individualized treatment and frequent observations has long been stressed. However, a

summary of recent research findings, along with certain principles, may offer possible explanations for responsiveness to make highly efficient lead with fewer side effects to resist both, coagulopathy as well as hypertension. The primary aim of this present work is to study pharmacological and synthetic aspects of the coumarin ring structure especially its combined analogues profile as an anti coagulant and anti hypertensive property.

## 2.5 CONCURRENT USE OF ANTIHYPERTENSIVE AND ANTICOAGULANT DRUGS

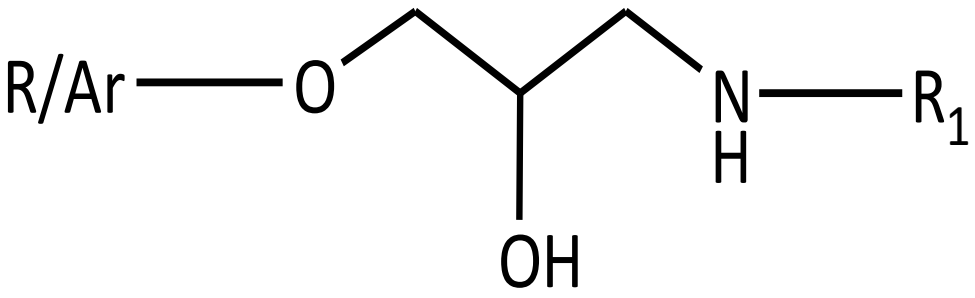
The main complications of hypertension, i.e. coronary heart disease, ischemic strokes and peripheral vascular disease (PVD), are usually related to thrombosis. Increasing evidence also suggests that hypertension fulfils the components of Virchow's triad, thus conferring a prothrombotic or hypercoagulable state, as evident by abnormalities of haemostasis, platelets and endothelial function. It therefore seems plausible that use of antithrombotic therapy may help prevent these thrombosis-related complications of hypertension. Indeed, hypertensive patients with an estimated 10-year CHD risk > or = 15% will have their cardiovascular risk reduced by 25% using antihypertensive treatment, but the addition of aspirin further reduces major cardiovascular events by 15%. Recent guidelines recommend the use of aspirin 75 mg daily for hypertensive patients who have no contraindication to aspirin.[35] Further, although systemic (arterial) elevations in blood pressure result in high intravascular pressure, the main complications of elevated blood pressure, coronary heart disease (CHD) events, ischemic stroke and peripheral vascular disease (PVD), are related to thrombosis. The association between elevated blood pressure and risk for stroke and CHD has a linear relationship, with increasing risk for higher blood pressures.[36] In addition it should be recognized that some of the complications related to elevated blood pressure, heart failure or atrial fibrillation, are themselves associated with thromboembolism.[37] Increasing evidence also points towards a prothrombic or hypercoagulable state conferred by the presence of elevated blood pressure, as evident by abnormalities of coagulation, platelets and endothelial function in such patients.[38-39]

It therefore seems plausible that use of antithrombotic therapy may be of particular benefit in preventing the thrombosis-related complications of elevated blood pressure.[40] The antithrombotic agent, acetylsalicylic acid (ASA), is established as an effective agent for secondary prevention in patients with proven occlusive vascular disease.[41] However, it is not recommended for primary prevention and it is unclear whether it has a role in patients with an increased risk of thrombotic complications such

as those with elevated blood pressure. Warfarin has also been found to be useful as thromboprophylaxis in patients with elevated blood pressure and atrial fibrillation (Segal 2001, SPAF II Study 1994); however, if blood pressures remain uncontrolled, such therapy carries significant risk, especially from intracranial haemorrhage.

Thus, since hypertension and thrombo-embolism are intricately involved in pathophysiological changes and progression of cardio-vascular diseases; antihypertensive drugs like propranolol and anticoagulant drugs like warfarin are frequently, combined in management of some types of hypertension. However, in clinical practice, it has been noted that prescribing, one drug with two different activities, improves patient compliance to the treatment instead of two drugs with two different activities resulting in better clinical outcome. *Therefore, in the present project, we attempted to synthesize a single drug molecule with antihypertensive activity of propranolol and anticoagulant activity of Warfarin (Coumadin).*

To achieve this, we attached alkylaminohydroxypropoxy side chain (responsible for antihypertensive activity of propranolol) to 4-Hydroxy coumarin nucleus (responsible for anticoagulant activity of warfarin). Several *resulting molecules were then, subjected to pharmacological evaluation, for both types of activities, to assess structure activity relationship and to ascertain the lead molecule.*

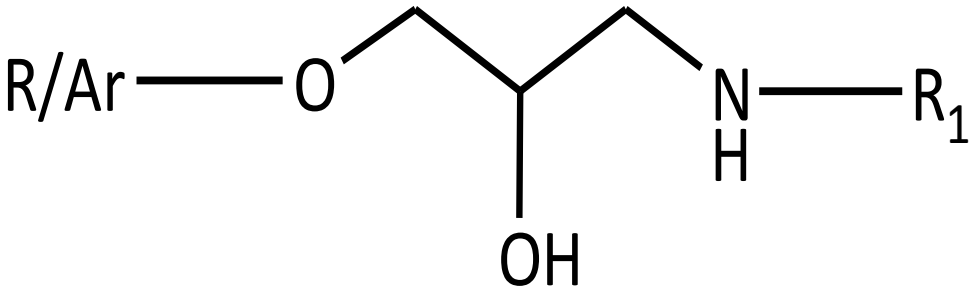



# OBJECTIVES OF THE PROJECT

## 3. OBJECTIVES OF THE PROJECT

Warfarin contains coumarin skeleton and propranolol contains isopropylamino-2-hydroxypropoxy side chain. Our aim was to synthesize antihypertensive agents with anticoagulant activity. Hence, the objectives of the present project were -

- *To synthesize a series of drug molecules having* coumarin skeleton with alkylamino-2-hydroxypropoxy side chain.
- *To subject the series of synthesized molecules to pharmacological evaluation for antihypertensive and anticoagulant activity so as to assess the structure activity relationship and to ascertain the lead molecule.*




# LITERATURE REVIEW

# 4. LITERATURE REVIEW

Epidemiologists in India and international agencies, such as the World Health Organization (WHO), have been sounding an alarm on the rapidly rising burdens of cardiovascular diseases (CVD) for the past 15 years. Cardiovascular diseases include hypertension, hyperlipidemia, heart failure, angina pectoris, arrhythmia etc. The reported prevalence of coronary heart disease (CHD) in adult surveys has risen 4-fold over the last 40 years (to a present level of around 10%), and even in rural areas the prevalence has doubled over the past 30 years (to a present level of around 4%). Cardiovascular disease is now the leading cause of death, accounting for 29% of all deaths in 2005, according to the WHO.[2] India, already the diabetes capital of the world with 32 million persons with diabetes, is projected to have 69.8 million in 2025. The count of "hypertensive" individuals is expected to rise from 118 million in 2000 to 214 million in 2025.

### 4.1 Antihypertensive Drugs

Hypertension is a multi factorial disease frequently associated with other cardiovascular problems. None of the antihypertensive drugs available can cure all cases of hypertension. Use of drugs acting on the adrenergic system, diuretics, calcium antagonists, nitro-vasodilators and so on, have not yet been abandoned, while new compounds are still being developed today.[42]

Van zwieten and his colleague concentrate on a considerable effort in neuropharmacological research have been invested in an attempt to localize the precise anatomical position of central α-adrenoceptors. However, localization in full detail has not been possible yet, owing to limitations of experimental methods. Central α-adrenoceptors are likely to be located within the brain stem.[43]

Mancia et al. (2000) worked on ACE inhibitors. Recent studies revealed that in diabetic hypertensive patients, administration of angiotensin-converting enzyme (ACE)-inhibitors or calcium antagonists can effectively lower blood pressure and prevent diabetes-related cardiovascular complications with no adverse metabolic effects.[44] Ishi and her colleague conclude that the systemic administration of

*cimetidine* with topical ocular timolol increases the degree of β blockade, resulting in a reduction of resting heart rate, intraocular pressure, and exercise tolerance.[45]

Prakash *et al.* (2000) worked on metoprolol and proved that it is effective in chronic heart failure. It has been shown in randomized trials to be associated with a striking reduction in all-cause mortality and hospitalization for worsening heart failure and a modest reduction in all-cause hospitalization.[47] Ferdinand and his colleagues worked in high-risk patients, including those with diabetes, renal insufficiency, left ventricular dysfunction, and atherosclerosis, and conclude that ACE inhibitors may have specific benefit in reducing cardiovascular morbidity and mortality.[48]

Same time Mc Gavin *et al.* (2002) proved that bisoprolol is a highly selective $\beta_1$ receptor antagonist but it does not have intrinsic sympathomimetic or membrane-stabilizing activity.[49] To treat hypertension and decrease mortality, Elly and his colleague worked on effect of exercise on heart. They conclude that regular exercise improves blood flow and helps to reduce the resting heart rate and blood pressure.[50] Kumar *et al.* (2011) investigated a new rout of administration of metoprolol to achieve sustained drug release kinetics and form oral dosage form.[51]

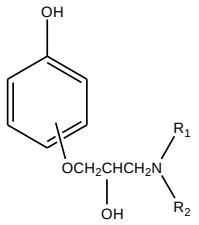
The hemodynamic consequences of long-term treatment with antihypertensive agents provide a rationale for potential complementary effects of concurrent therapy with two or more drugs. The simultaneous use of drugs with similar mechanisms of action and hemodynamic effects often produces little additional benefit. However, concurrent use of drugs from different classes is a strategy for achieving effective control of blood pressure while minimizing dose-related adverse effects.[46]

### 4.2 β-BLOCKERS

#### 4.2.1 Pharmacological Aspects of B-Blockers and Side Chain

Hieble *et al.* synthesized a series of compounds which are derivative of alkylaminohydroxypropoxy and hydroxyl substituted on aromatic ring (17). [52] Powell and Slater described the activity of dichloroisoproterenol (18) and it was the first β blocker reported in 1958.[53] Nakagawa *et al*. (1971) synthesized antiarrhythmic 5-(3-

alkylamino-2-hydroxypropoxy)-8-nitro-3,4-dihydrocarbostyrils (19) derivatives.[54] After getting success, Nakagawa and his colleague (1976) synthesized antiarrhythmic and antianginal compounds having 5-(3-Alkylamino-2-hydroxy)propoxy side chain of 3,4-dihydrocarbostyrils derivative (20). They also performed activity of synthesized 5-(3-Alkylamino-2-hydroxy) propoxy-8-hydroxy-3,4-dihydrocarbostyrils (21) derivatives and proved antihypertensive activity.[55] Black et al. performed activity of many compounds, they found that pronethalol (22) and propranolol (23) has β antagonistic activity.[56] Kaiser *et al.* (1977) reported that –$OCH_2$– group is responsible for binding at β-receptor site for agonistic or antagonistic activity.[57] Then research was carried out for analyze other parameters like metabolism and excretion of drug through body. Riddell and his colleagues proved that the aryl group affects the absorption, excretion and metabolism of the β blockers.[58]

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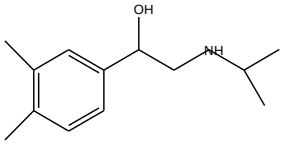


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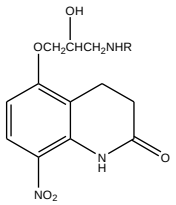


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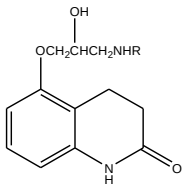


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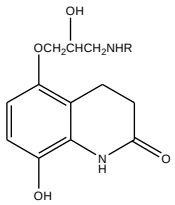


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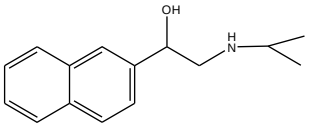


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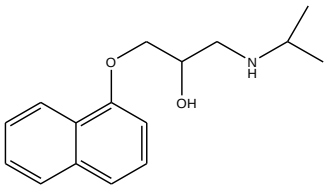


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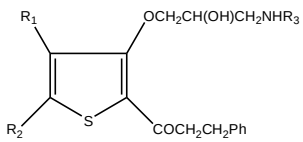
Discovery of propranolol cause increase research of cardiovascular system. Researcher modified aromatic skeleton and performed activity of synthesized compounds. Binder *et al.* (1982) synthesized 3 substituted thiophene derivatives (24) and performed antiarrythmic activity induced by calcium chloride in mice.[59] Chalina *et al.* (1987) synthesized 5,8 bis(3-Alkylamino-2-hydroxy)propoxy-1,4-dihydronaphthalene (25) and pharmacological screening showed hypotensive and β-adrenoceptor blocking activities, as well as low toxicity.[60] Cai *et al.* (1990) synthesized 3-[4-[(3-alkylamino-2-hydroxy) propoxy] phenyl (benzyl)]-substituted-4(3H)-quinazolinones (26) derivatves and showed increase the tolerance of mice to hypoxia.[61] Lots of research works on alkylaminohydroxypropoxy side chain prove that it plays important role for β-blocking activity. Racanska *et al.* (1990) tried to find relationship between the chemical structure and pharmacological activity of some newly synthesized alkylesters of 4-[(2-hydroxy-3-alkylamino) propoxy]phenylcarbamic acids derivatives (27). They conclude that 4-substituted derivatives of aryloxypropanolamines had antiisoprenaline (beta-adrenolytic) and local anesthetic (membrane stabilizing) activity.[62] Discovery of other antihypertensive agents and minimize their drawbacks was excellence research during that time.

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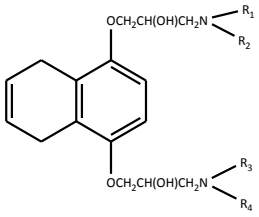


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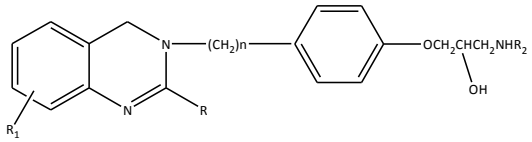


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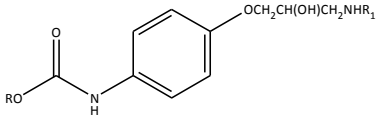


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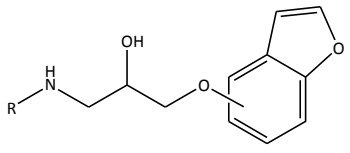
Ondriasova *et al.* (1992) compared the potency of five potential β-adrenoceptor blocking drugs like alkylesters of 4-[(2-hydroxy-3-alkylamino) propoxy]phenylcarbamic acid derivative and eight calcium channel blockers like nifedipine, nimodipine, niludipine, nitrendipine, verapamil etc. to inhibit platelet aggregation and to perturb liposomal membranes prepared from platelet lipids.[63]

### 4.2.2 Chemical Aspects of β-Blockers and Side Chain

Turner *et al.* (1968) synthesized 4(5,6, and 7)-(3-Alkylamino-2-hydroxy-1-propoxy) benzofurans (28) and indoles from mixture of 4-hydroxybenzofuran, epichlorohydrin and piperidine. Further reaction was amination to produce final products.[64] Troxler *et al.* (1971) synthesized 4-[2-Hydroxy-3-(alkylamino)propoxy]-2-(methoxymethyl)indoles (29) by treating 4-hydroxy-2-(methoxymethyl)indole with epichlorohydrin in aqueous dioxane in the presence of sodiumhydroxide. Synthesized compounds possessed β-blockling and antiarrithmic activity.[65] Sato *et al.* (1972) synthesized twenty-three tropone derivatives from methoxytropones, p-aminophenol followed by reaction with epibromohydrin and followed by amination. Final compounds showed adrenergic blocking, hypotensive, and vasodilating activity.[66]

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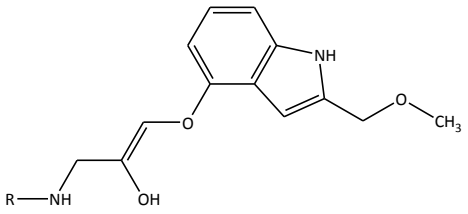


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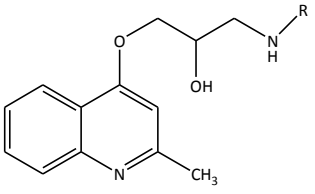
Ahmed *et al.* (1998) performed reaction of 4-hydroxyquinaldine/6-bromo-4-hydroxyquinaldine/6-fluoro-4-hydroxyquinaldine with epichlorohydrin followed by amination to form 4-(3-alkylamino-2-hydroxy-1-propoxy)quinaldines (30) and 6-haloquinaldines derivatives. Synthesized compounds were evaluated for their antihypertensive activity and beta blocking activity against chronotropic response to adrenaline. The iso-propyl and tert-butyl analogs showed marked decrease in blood pressure, the iso-propyl analogs were more potent than propranolol. All synthesized compounds exhibited marked decrease in heart rate, cardiac output and force of contraction. This work was continued for multiple pharmacological activities rather than single antihypertensive activity.[67] Ahmed *et al.* (1998) synthesized 6-hydroxy 3-substituted, 6-(alkylamino(hydroxy)propoxy)-2H-1,2,4-benzothiadiazine-1,1-dioxide-7-sulfonamides. They have synthesized 6-Hydroxy-3-(substituted)-7-sulfamoyl-3,4-dihydro-1,2,4-benzothiadiazine 1,1-dioxides by condensing 3-aminophenol-4,6-disulfonamide with different aldehydes. 6-(3-Alkylamino-2-hydroxypropoxy)-7-sulfamoyl-2H-1,2,4-benzothiadiazine 1,1-dioxides were obtained by reacting 6-hydroxy-7-sulfamoyl-2H-1,2,4-benzothiadiazine 1,1-dioxide with epichlorohydrin followed by addition of different alkylamines. These compounds being a hybrid of benzothiadiazine (diuretics) and aryloxypropoxyamines (β-adrenergic blockers) are expected to have both these properties and may be useful as better hypotensive compounds.[68]

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## 4.3 ANTICOAGULANTS

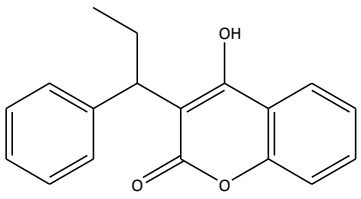
### 4.3.1 Pharmacological Aspects of Anticoagulants

The story of the coumarin anticoagulants generally is traced back to the early 1920. When the "sweet clover disease" showed up in North Dakota, Alberta and Canada, many scientist made in surprise for increase bleeding in cattle by sweet clover plant. Vogel et al. (1920) showed that coumarin is a widely occurring secondary metabolite that occurs naturally in several plant families. Coumarin name of this chemical family is derived from *Coumarouna odorata Aube (Dipteryx odorata) tonka beans*, from which coumarin was isolated first time.[69] Professor Link and his students (1934) developed reliable bioassay method of anticoagulation at the Wisconsin Agriculture Experiment Station. Campbell and Link (1939) ingested spoiled sweet clover silage in cattle and produced hemorrhagic disorder. Finally they identified the hemorrhagic agent as bishydroxycoumarin derivative and named as dicumarol (16). After discovery of dicumarol many scientists worked on coumarin derivatives to discover other anticoagulant agents. Ikawa and his colleague (1942) synthesized warfarin (15) in the laboratory of Professor Link and proved its high anticoagulant activity in rats, At that time it's extensive application found as a rodenticide rather than anticoagulant. Warfarin was introduced into clinical anticoagulant therapy in 1952.[70]

Shepard *et al.* (1944) studied the effect of synthetic vitamin K on the prothrombinopenia induced by salicylate in man. The prothrombinopenia resulting from dicumarol, which occurs only in vivo and after a certain latent period, due to the degradation of the dicumarol to 2 moles of salicylic acid. The same effect is obtained when salicylates are administered. It is, therefore, essential to determine the prothrombin level when salicylate therapy is used.[71] Hais *et al.* (1951) worked on degradation of 4-hydroxycoumarin by using paper chromatography in urine and blood. . Ammonia-water-butanol or octanol system was used for the detection and identification of pelentan, dicoumarol and some of their degradation products in blood and urine.[72]

After successive discovery of warfarin as anticoagulant, scientist tried to found new applications of coumarin. Lacharme *et al.* (1952) worked on derivatives of 4-hydroxycoumarin as inhibitors of germination and growth of plants. So, they gave new direction to agriculture field by performed above research.[73] It was the time where parallel research was going in both medical and agricultural field. Martius *et al.* (1953) worked to discover mechanism of action of dicumarols and related compounds. 3,3'-Methylenebis (4-hydroxycoumarin) and related compounds, they proved that compounds inhibit aerobic phosphorylation in mitochondria of rat.[74] Henrik *et al.* (1954) worked to determine levels of vitamin K1 in blood and various organs of chicks and rats after administration of massive doses of K1. In chicks a massive dose of vitamin K1 injected intravenously (in colloidal suspension prepared with the aid of Tween 80) disappeared gradually from the blood stream and was deposited in the liver, spleen, and lungs. None was found in the bile. They proved that massive doses of given intravenously accumulated in the liver and spleen in rats. Only small amounts were found in the lungs and kidneys on the day after injection. After 28 days about 11 % of the dose was still present in liver and spleen.[75]

Hellemans *et al.* (1963) determined the survival time of prothrombin and factors VII, IX and X after completely synthesis blocking doses of coumarin derivatives. The synthesis of prothrombin and of factors VII, IX, and X in dogs was completely blocked with 3-(1-phenylpropyl)-4-hydroxycoumarin (32), bis(4-hydroxy-3-coumarinyl)ethyl acetate (33), or 3-(α-acetonyl-p-nitrobenzyl)-4-hydroxycoumarin (34). They concluded that the half-lives of prothrombin, 41.0; cloting factor VII, 6.2; IX, 13.9; and X, 16.5 hrs.[76] Giacomello *et al.* (1965) studied SAR of cholramphenicol they conclude that -$COCHCl_2$ group is essential for antibacterial effect. So they synthesized dichloroacetic acid derivatives of coumarin and performed antibacterial activity of synthesized compounds.[77]



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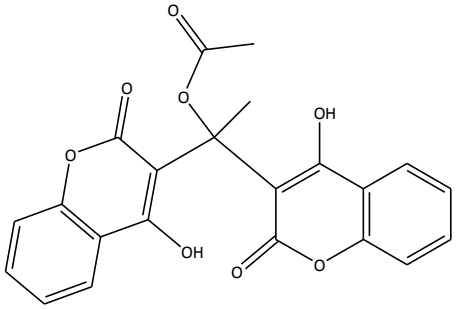

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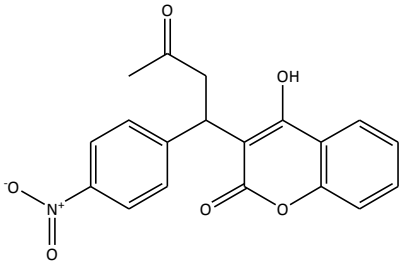

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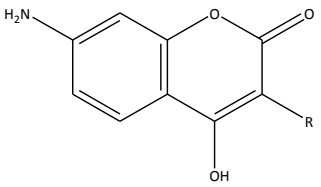
Rowe *et al.* (1967) studied the effect of sex and age on the response to warfarin in a noninbred strain of mice. They observed that marked sex difference in susceptibility to warfarin was found in 6-months old gray mice, males being more susceptible than females. No sex difference was found in young mice which were 6-weeks old.[78] Ichikawa *et al.* (1969) reported antibacterial activity of 3-substituted-7-amino-4-hydroxy-coumarin derivatives (35).[79]

Perone *et al.* (1972) survey that large quantities of coumarin were used in the food industry, mostly associated with vanillin, for flavoring chocolates, baked goods, and in cream soda-flavoured beverages.[80]

Olson *et al.* (1975) studied on mode of action of vitamin K in regulating prothrombin synthesis. They developed hypothetical model in which vitamin K acts to regulate the biosynthesis of prothrombin and other vitamin K dependent clotting proteins at the ribosomal level are presented. The model provides a regulatory protein which binds vitamin K and the 4-hydroxy-coumarin drugs at separate sites and associates with both the 60S and 40S particle to ribosome and has a recognition site for the prothrombin messenger RNA which is attached to the 40S subunit of the ribosome.[81]

In U.S. Beriger reported insecticidal activity of synthesized active 3-N-(4-trifluoromethylphenyl) carbamoyl-4-hydroxycoumarin (36) in 1975. Ritschel *et al.* (1977) studied the pharmacokinetics of coumarin and its 7-hydroxy-metabolites upon intravenous and peroral administration of coumarin in man. They conclude that glucuronide conjugation is more preferred path for excretion.[82]

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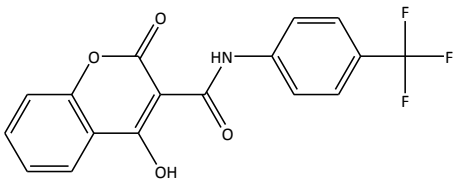


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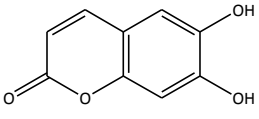
Sekiya *et al.* (1982) studied the effects of coumarin and its derivatives on rat platelet lipoxygenase and cyclooxygenase activities. Esculetin (37) was found to inhibit the lipoxygenase more strongly than the cyclooxygenase; its IC50 was 0.65 mM for platelet lipoxygenase and 0.45 mM for platelet cyclooxygenase. Esculin (the 6-glucoside of esculetin) and umbelliferone (38) also selectively inhibited the lipoxygenase, though less strongly (IC50 = 290 and 500 mM, respectively). 4-Hydroxycoumarin and coumarin had no inhibitory effect on either enzyme at concentrations up to 1 mM. The mechanism of the lipoxygenase inhibition by esculetin was non-competitive.[83]

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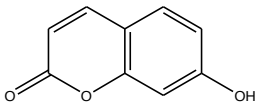


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Marshall et al. (1987) stated that coumarin has been recommended for treatment of a number of clinical conditions, including high protein edema and brucellosis. It is currently undergoing clinical trials for treatment of lymphoedema following breast cancer treatment and in treatment of lung and kidney cancer and of melanoma alone or in combination with cimetidine.[84-90]

Chahinian (1989) proved anticancer activity of coumarin derivatives. They also proved their mechanism of action and conclude that they act by disorganizing the mitotic spindle microtubules in cells, leading to the random distribution of chromosomes at metaphase. Because of their low toxicity and simple chemical structure, there is potential interest to explore combination of antimitotic coumarins with other chemotherapeutic agents to improve efficacy and lower toxicity of anticancer drugs.[91]

Egan *et al.* (1990) reported use of coumarin. They found that it is used as odour-enhancer to achieve a long-lasting effect when combined with natural essential oils such as lavender, citrus, rosemary and oak moss. Coumarin is used in tobacco to enhance its natural aroma. It is also applied in large quantities to give pleasant aromas to house - hold materials and industrial products or to mask unpleasant odours.[92] Myszka *et al.* (1991) discovered fatty acid-binding protein (FABP) as a warfarin receptor in rat liver by photo affinity labeling method. They conclude that the high concentration of FABP was a major hepatic receptor responsible for the uptake and/or transport of various oral 4-hydroxycoumarin in liver.[93]

Lake *et al.* (1993) first time proved hepatotoxicity of coumarin by oxidase enzyme inducers in the rat, and therefore many scientists worked on drawback of coumarin which is widely used as flavoring agent in food industries. Finally, the use of coumarin as direct food additive has been suspended in the United States.[94] Further work was continued by Marshall *et al.* (1994). They reported that coumarin and 7-hydroxycoumarin have immunomodulatory and antitumor activity.[95] Chang *et al.* (1995) worked on esculetin, umbelliferone and 7-hydroxy-4-methyl coumarin and proved that all are strong xanthine oxidase inhibitors with IC50=20.91, 43.65 and 96.70 mM respectively.[96]

Mileti *et al.* (1995) tried to form sustain release drug delivery system of Coumadin to decrease its severe side effects such as excessive hemorrhage and necrosis. They decreased the dosage of Coumadin by maintaining an effective threshold level of the drug using a hydroxyapatite ceramic drug delivery system.[84-97] During this era, many scientists worked to prove other uses of coumarin above its highly severe side effects. Galabov *et al.* (1996) found antiviral activity of esculetin. They showed that ten samples exhibited a marked inhibitory effect on Newcastle disease virus replication in cell cultures. Antiviral activity was proved on different species of virus like, picorna, orthomyxo, paramyxo, and herpes families.[98]

Hoult *et al.* (1996) stated that many coumarin derivatives have been found in varieties of plants; to date more than 1300 green plants have been identified, principally as secondary plant metabolite.[99] Botanical sources of coumarin are widely distributed in nature, which pull many scientists to take effort to prove its different pharmacological activity. Weber *et al.* (1998) reported antitumor activity of coumarin, 7-hydroxy-coumarin and its glucuronide in several human tumor cell lines. Both compounds inhibited cell proliferation of a gastric carcinoma cell line, a colon-carcinoma cell line, a hepatoma-derived cell line and a lymphoblastic cell line.[100] Furthermore investigation on anti tumor activity is carried out by Mousa *et al.* (2000). They reported effect of coumarin on tissue factor VIIa, to be a major factor in the regulation of angiogenic growth properties of tumor cells.[101]

Zaton *et al.* (2000) studied on binding of drugs to human serum albumin and determined drug distribution through systemic circulation. They found that structural features of 4-hydroxy-coumarin, 3-acetylcoumarin, coumarin**,** benzylthiouracil, propyluracil, thiouracil, chromone and chromanol are very similar to warfarin, propylthiouracil and cromoglycate. Because of structural similarity, these compounds were competitively displaced by warfarin at their primary binding sites on seroalbumin.[102] Structural similarities and protein binding sites are new area of interest to found more toxic effects of coumarins and its derivatives. Parallel to this, Masamoto *et al.* (2001) investigated contact sensitization of 11 simple coumarins. Esculetin, 4-methylesculetin, and daphnetin were found to be strong sensitizers, and 4-hydroxy-coumarin to be a moderate sensitizer. The results suggest

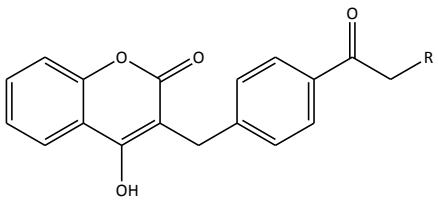
that the introduction of hydroxy group, especially adjacent substitution at the 6, 7, and 8 positions of the coumarin ring with two hydroxy groups, may play an important role in exhibiting the contact sensitization activity. The cross-reactivity was observed between esculetin and 4-methylesculetin, esculin or isoscopoletin, and also between daphnetin and 4-methylumbelliferone or umbelliferone. It is interesting to note that guinea pigs, which had a weak sensitivity to umbelliferone, showed a strong cross-reactivity to daphnetin, while those, which had a weak sensitivity to daphnetin, showed a weak cross-reactivity to umbelliferone. It is assumed that a skin-protein conjugation at 5 or 6 positions of the coumarin ring is important to elicit the cross-reactivity of esculetin or daphnetin groups.[103]

Chen *et al.* (2001) worked on coumarin group of antibiotics, such as novobiocin, coumermycin A1 and clorobiocin; those are potent inhibitors of DNA gyrase. They isolated these antibiotics from various streptomyces species and all possess a 3-amino-4-hydroxy-coumarin moiety as their structural core. They conclude that the coumarin moiety was derived from L-tyrosine, probably via a β-hydroxy-tyrosine intermediate.[104] Coumarine derivatives were extracted from different bacterial species. Sun *et al.* (2002) extracted 6-methoxy-7-hydroxycoumarin, β-sitosterol and 5,7-dihydroxycoumarinI from Morus alba. All structures were confirmed by by EI-MS, IR, 1H NMR, UV analytical techniques. They determined anti asthmatic and anti diuretic activity of 6-methoxy-7-hydroxycoumarin.[105]

Kamat *et al.* (2004) discovered the mechanism of interaction of four coumarin derivatives with bovine serum albumin using spectrofluorometric technique. It was concluded that the coumarin ring plays major role in interaction. Stern-Volmer plots and parachor values of coumarin derivatives explain the quenching mechanism.[106] Coumarin derivatives became the centre of interest for researchers for its anti tumor activity. Mazumder *et al.* (2004) showed promising antitumor activity of 3-hydroxy coumarin. Treatment with it prolonged the life span of mice as well as decreased their tumor volume and viable ascitic cell count. All the tested complexes exhibited mild to moderate antibacterial activity.[107]

Ghate *et al.* (2005) synthesized coumarinyl ethers having chromone, benzofuranyl and 4-hydroxy coumarins and tested them for analgesic and anti-inflammatory activity in rat. These newly synthesized compounds were found to produce less toxicity and less ulcerogenic activity.[108] Different isolation methods to collect coumarin derivatives were being established. First time Yuan et al. (2005) isolated 6-methoxyl-7-hydroxyl coumarin and 7-hydroxyl coumarin from roots of Ipomoea batatas Lam. variety Simon.[109] Different synthetic methods were also produced to get various coumarin derivatives. Druzgala *et al.* (2005) prepared 3-benzyl-4-hydroxy-coumarin derivatives as inhibitors of vitamin K epoxide reductase and posses anticoagulant activity. The activity (39, 40) was evaluated using cow liver microsomes.[110]

Kontogiorgis *et al.* (2005) stated the anti inflammatory and antioxidant activity of novel coumarin derivatives. Some of them were found in vitro to inhibit lipid peroxidase and strongly scavenging superoxide radicals. Compd. 3 was found as a potent inhibitor of cyclooxygenase-1 and the yeast-induced rat paw edema. It was tested against adjuvant-induced arthritis and found to significantly protect the rats from it.[111] More insight into pharmacological uses of coumarin and its derivatives was continued by researchers. Anamik *et al.* (2005) synthesized series 4-hydroxy derivatives and screened them in vitro for anti-HIV activity against HIV-1(IIIB) and HIV-2(ROD) virus strains. Compound (41) was found to be the most active.[112]

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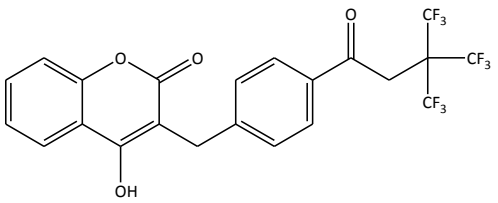


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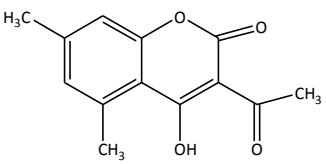


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Coumarin got attention as an antifungal activity by Brooker *et al.* (2005). They synthesized halogenated (brominated, chlorinated, iodinated) coumarins and evaluated for antifungal activity against soil-borne plant pathogenic fungi (against Macrophomina phaseolina, Pythium spp., Phytophthora spp.) in soybean seed germination and in early development. Halogenated coumarin compounds have higher antifungal activity as 4-hydroxy-coumarin alone. Halogenated coumarins and 4-hydroxy-coumarin showed 100% fungal inhibition for 21 days. Phytotoxicity tests were negative.[113]

Cheng *et al.* (2005) discovered a novel application of coumarin compounds in preparing medicine for inducing directional differentiation of neural stem cell by treating demyelination or spinal cord injury.[114] There were a number of reports that natural and synthetic coumarin derivatives posse's antimicrobial activity. Kulkarni *et al.* (2009) worked on in-vitro antimicrobial studies of Co(II), Ni(II), and Cu(II) complexes with Schiff bases of formyl coumarin derivatives. The Schiff bases and their complexes were screened for antibacterial (Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, and Salmonella typhii) and antifungal (Aspergillus niger, Aspergillus flavus, and Cladosporium) activities by Minimum Inhibitory Concentration (MIC) method. The redox behavior of the complexes was studied using cyclic voltammetry.[115]

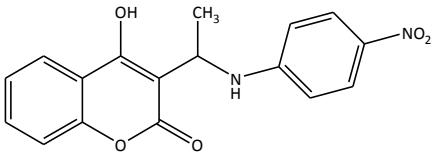
Bhattacharyya *et al.* (2009) found that isolated Scopoletin from Gelsemium sempervirens (Fam: Loganiaceae) has been reported to have anti-cancer potentials. So their colleague tried to synthesize 4-Methyl-7 hydroxy coumarin from resorcinol. They screened synthetic compounds for anti-cancer potentials and has been evaluated in vivo on DMBA (7,12-Dimethylbenz[a]anthracene) induced skin cancer in mice by analyzing results of several cytogenetic endpoints, Comet assay, and fluorescence activated cell sorting.[116]

Yousef *et al.* (2010) prepared 4-hydroxy coumarin-3-thiocarbohydrazon by the reaction of 4-hydroxy coumarin with dithiocarbohydrazide in molar ratio 1:1, and performed some biochemical parameters and histological studies in serum, liver and kidney of rats. They observed the effect of Aspergillus niger and Candida albicans on

the radial growth. The results of this study prove that the complex at low dose has a better effect than the complex at high dose and the ligand at both high and low doses has no effect on the biochemical analysis in serum, liver, kidney tissues and histological examination in rats.[117]

Vukovic *et al.* (2010) worked on synthesis and antioxidant properties of novel imino and amino derivatives of 4-hydroxy coumarins (42, 43). Series of imino and amino derivatives of 4-hydroxy coumarins were synthesized via conventional and microwave promoted procedure and evaluated for antioxidant potential through different in vitro models such as free radical scavenging activity, linoleic acid emulsion model system, reducing power assay and phosphomolybdenum method. All prepared compounds possess good antioxidant activity and among them p-nitro-Ph derivative possesses radical scavenging activity which is comparable to BHT, while the best reducing power was observed in a case of benzyl amino compound.[118]

Shen *et al.* (2010) worked on hydroxycoumarin Derivatives as novel and potent α-Glucosidase Inhibitors. Among all hydroxycoumarin derivatives studied, compounds 44 and 45 exhibited the highest activities, were specific inhibitors of α-glucosidase, and could be exploited as the lead compounds for the development of potent α-glucosidase inhibitors.[119]

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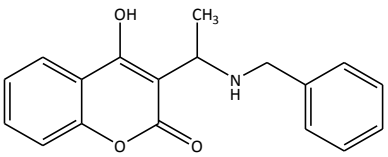


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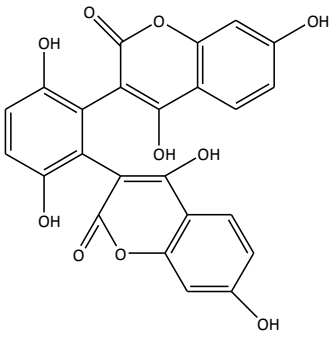


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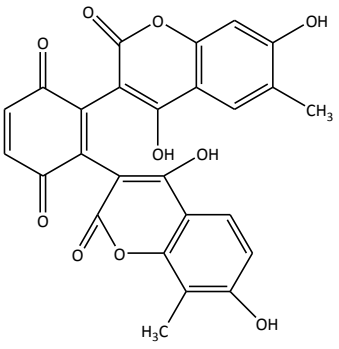


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### 4.3.2 Chemistry Aspects of Coumarin

Seidman *et al.* (1950) synthesized anticoagulant and rodenticide compounds like, 3-(α-acetonylbenzyl)-4-hydroxycoumarin in 67% yield by refluxing 4-hydroxycoumarin and 4-phenylbutan-2-one in presence of a mixture of dioxane and piperidine.[120] Hais *et al.* (1951) first time developed a paper chromatography technique for the analysis of 4-hydroxycoumarin derivatives. Here $NH_3$-$H_2O$-butanol or octanol system was used for the detection and identification of pelentan and dicoumarol and some of their degradation products in blood and urine.[121] Knobloch *et al.* (1953) studied infrared spectra of many compounds and based on IR spectra conclude that chromone derivative may be responsible for anticoagulant activity.[122] Klosa *et al.* (1956) synthesized 4-Acetoxycoumarin, 4-propionyloxycoumarin, and 4-butyryloxycoumarin; they were subjected to the Fries reaction with several metal halides as catalysts and 4-hydroxycoumarin as starting phenol.[123]

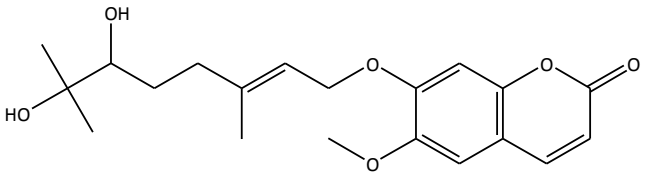
Indian scientist Bose *et al.* (1960) developed new method for synthesis of 4-hydroxycoumarin. They synthesized 4-hydroxy coumarins by heating diaryl malonates with equimolor moles of corresponding malonic acid in the presence of about anhydrous $ZnCl_2$ and $POCl_3$.[124] Schroeder *et al.* (1960) proved mechanism of formation of warfarin from 4-hydroxy- coumarin and β-anilinobenzylacetone. They conclude that Schiff base intermediates showing enhanced reactivity in the Michael reaction.[125]

Checchi *et al.* (1966) synthesized aminomethylene derivatives of 4-hydroxycoumarin by aminolysis of 3 substituted derivatives of pyrano(3,2-c)benzopyran-2,5-dione . They also prepared Schiff bases from 3-formyl-4-hydroxy-coumarin and the corresponding primary amines.[126] Reiche *et al.* (1966) performed condensation reaction of alkynols (1-phenylbut-3-yn-1-ol) with 4-hydroxycoumarins in mixture of acetic acid with sulphuric acid or AcOH-BFborontrifloride yielded anticoagulants of the warfarin type in 70-80% yields. This was first attempt to use alkynol for synthesis of anticoagulant compounds.[127]

Kumar *et al.* (1966) synthesized N-thiocarbamyl derivatives of 3-amino-4-hydroxy coumarin by 3-Amino-4-hydroxycoumarin were refluxed with an equivalent amount of aryl isothiocyanate in ethanol to give final product.[128] Pozetti *et al.* (1970) were identified and characterized 4-hydroxyderivatives by thin-layer chromatography They used silica gel HF254-cellulose (1:1) and silica gel G-cellulose (1:1) as stationary phase with mobile phase as the following 3 solvent systems: 20:3 C6H6-AcOH; 20:20:6.6:0.7 Me2CO-AcOEt-petroleum ether-H2O; 5:4:1 C6H6-AcOEt-AcOH. The plates were developed by spraying a solution of diazotized p-nitroaniline or 0.5% KMnO4, and examined under UV light.[129]

Kutznetsova *et al.* (1972) were isolated two compounds of coumarin type from H. pedicellatum and IR spectrum was characterized by absorption bands at 3420 and 1710 $cm^{-1}$, uv spectrum by absorption max. at 230, 252, 297, and 345 nm; the compound proved to be 6-methoxy-7-[(6,7-dihydroxy-3,7-dimethyl-2-octenyl)]oxy-coumarin (46) by other analytical datas.[130]

Mitra *et al.* (1982) reported [3,3]Sigmatropic rearrangement of allyl ethers of 3-hydroxycoumarin (47) and synthesized derivatives of 48$^{th}$ compound.[131]

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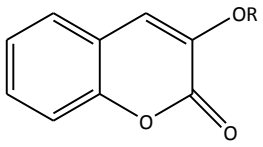


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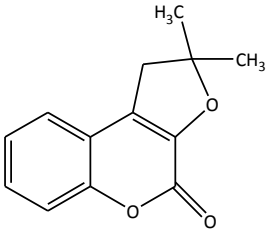
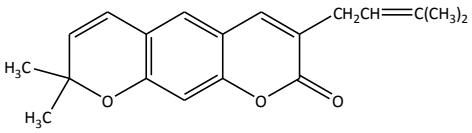



48

Swaroop *et al.* (1983) developed a convenient method for the construction of 3,3-dimethylallyl unit at C-3 of coumarin nucleus and utilized in the synthesis of the title compound (49). The synthetic strategy involves condensation of 3-formyl-7-

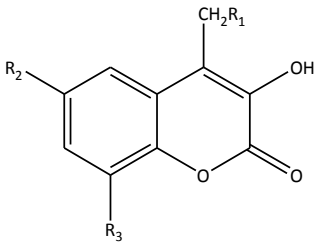
benzyloxycoumarin with acetone under acid-catalyzed condition, followed by catalytic hydrogenation, Grignard reaction with MeMgI, and dehydration. Finally, condensation of 3-(3, 3-dimethylallyl)-7-hydroxycoumarin with 4,4-dimethoxy-2-methylbutan-2-ol affords the desired compound.[132]



49

Kappe *et al.* (1995) synthesized alkylhydroxyquinolinones from acylhydroxyquinolones using Zn powder (particle size <45 mm) in acetic acid and hydrochloric acid.[133] Miky *et al.* (1995) performed Mannich reaction of 3-hydroxycoumarin derivatives with various amines in the presence of formaldehyde gave corresponding products(50).[134]

Zhang *et al.* (2004) discovered unique reaction of 4-hydroxy-2H-1-benzopyran-2-one with 2,5-cyclohexadiene-1,4-dione and pyridine gave [2,5-dihydroxy-6-(4-hydroxy-2-oxo-2H-1-benzopyran-3-yl)phenyl]pyridinium salt (51). The structures of these compound were detected by IR, MS(ESI), 1H NMR, 13C NMR, the single-crystal X-ray diffraction.[135]



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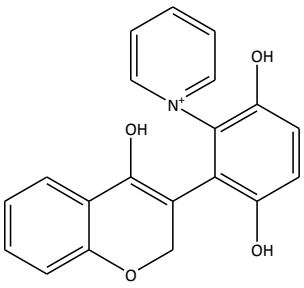


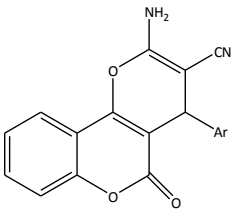
51

Fodorv *et al*. (2005) were synthesized benzopyran derivatives in good yields by the reaction of tris[2-(chloromethyl)phenyl]bismuth diacetate and [2-(halomethyl) aryl]lead triacetate with phenols and naturally occurring 4-hydroxycoumarins in the presence of bases according to a three-step one-pot procedure. Products thus

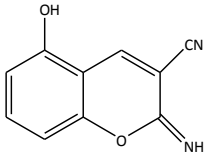
prepared included derivatives of 6H,11H-[2]benzopyrano[4,3-c][1]benzopyran-11-one, 1,3-dimethoxy-6Hdibenzo[b,d]pyran.[136]

Shestopalov *et al.* (2005) synthesized 2-amino-5-oxo-4,5-dihydropyrano[3,2-c]chromenes (52) from 4-hydroxycoumarin, carbonyl compounds, and malononitrile or alkyl cyanoacetates in ethanol in the presence of triethylamine as a catalyst.[137]

Volmajer *et al.* (2005) performed Knoevenagel reaction between 2-hydroxybenzaldehydes and active methylene compounds (malononitrile and ethylcyano acetate) produced iminocoumarins (53).[138]



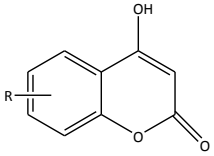

52




53

Abhijit *et al.* (2006) were synthesized 4-Aryl- and 4-alkylaminocoumarins by reaction of 4-hydroxycoumarin with amines under microwave irradiation in solvent-free conditions in good to excellent yields.[139]

Olga *et al.* (2006) discovered novel short-step methodology for the synthesis of coumarin in good yield starting from an activated precursor, the N-hydroxysuccinimide ester of O-acetylsalicylic acid. The procedure is based on a tandem C-acylation-cyclization process under mild reaction conditions. The structure of 3-methoxycarbonyl-4-hydroxy coumarin has been established by X-ray diffraction analysis and its geometry was compared with optimized parameters by means of DFT calculations.[140] Gao *et al.* (2008) investigated the preparation of 4-hydroxycoumarin derivatives (54) via heterocyclization of phenol derivatives with Meldrum's acid. [141]




54

### 4.3.3 Synthetic Methods of Coumarin

Coumarin and its derivatives were synthesized by many researchers, using different methods. Pechmann[142] synthesized coumarin from phenol. Several methods are reported for the synthesis of 4-Hydroxy coumarins and their 4-Hydroxy substituted derivatives namely:

1 Perkin[143]

2 Anschutz method [144]

3 Pauli Lockemann synthesis [145]

4 Sonn's synthesis [146]

5 Mentzer's synthesis [147]

6 Robertson synthesis [148]

7 Ziegler and Junek method [149]

8 Garden's method [150]

9 Shah, Bose and Shah's method [151]

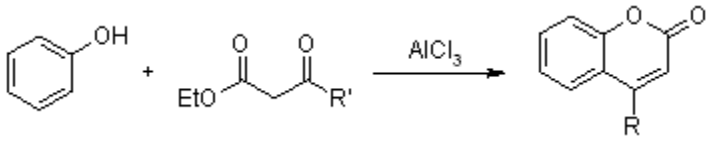
10 Kaneyuki method [152]

11 Resplandy's method [153]

12 Jain, Rohatagi and Sheshadri's method [154]

13 Shah, Bhatt and Thakor's method [155]

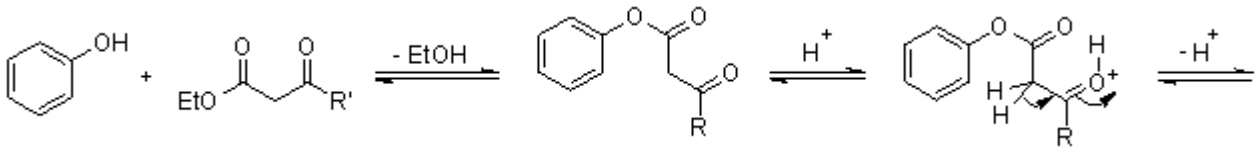
**Pechmann Condensation**



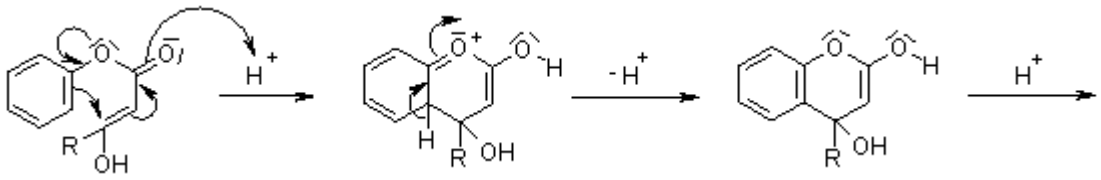

It is a Condensation reaction that allows synthesis of coumarin by reaction of phenol with β-keto esters.

**Mechanism of Pechmann Condensation**

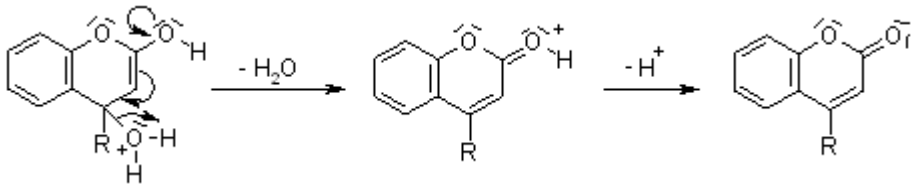
**Step 1**




**Step 2**

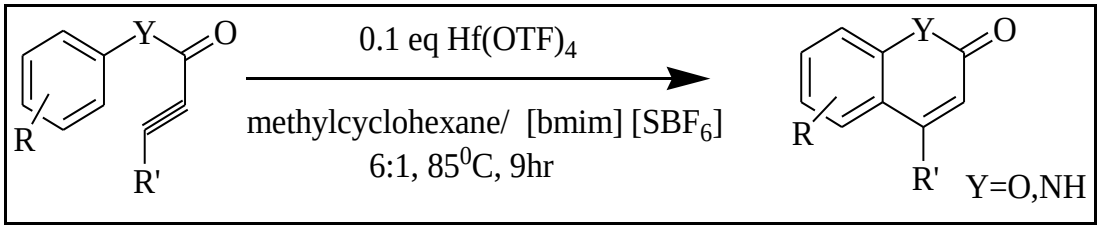



**Step 3**

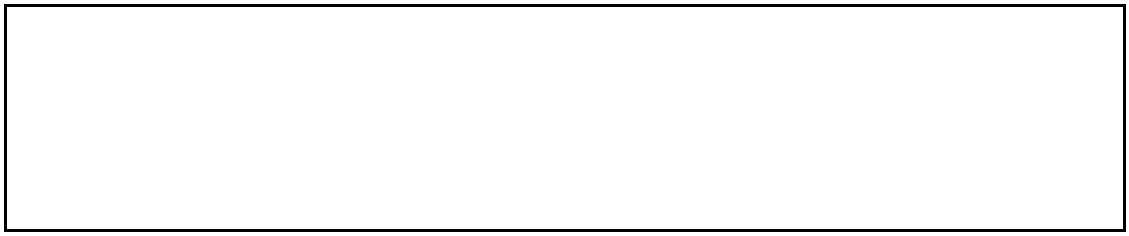


Shah *et al.*[151-155] have prepared 4-Hydroxy coumarin derivatives by condensation of different phenols with malonic acid in the presence of zinc chloride and phosphorous oxychloride. The method is useful as single step preparation of 4-Hydroxy coumarin derivatives substituted in benzenoid part and synthesized in good yield.

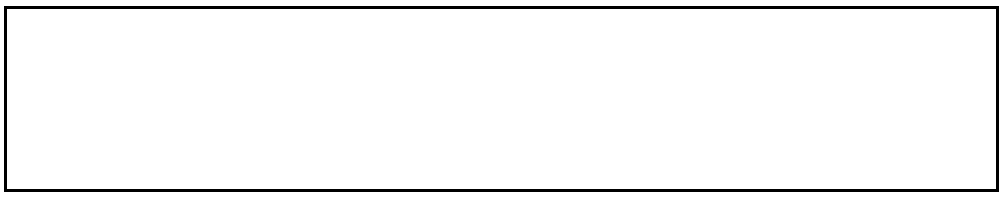
Recently many researchers[156-187] have reported synthetic strategies for 4-Hydroxy coumarin.




The employment of hydrophobic ionic liquids dramatically enhanced the activity of metal triflates in Friedel-Crafts alkenylations of aromatic compounds with various alkyl-and aryl-substitutedalkynes.[188]

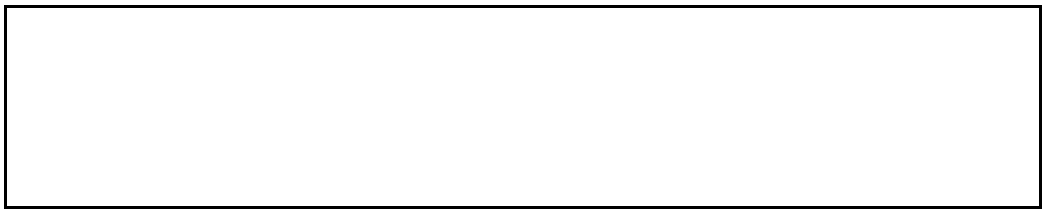


Arylpropionic acid methyl esters having a MOM-protected hydroxy group at the *ortho* position underwent hydroarylation with various arylboronic acids in methanol at ambient temperature in the presence of a catalytic amount of CuOAc, resulting in the formation of 4-arylcoumarins in high yields after the acidic workup.[189]

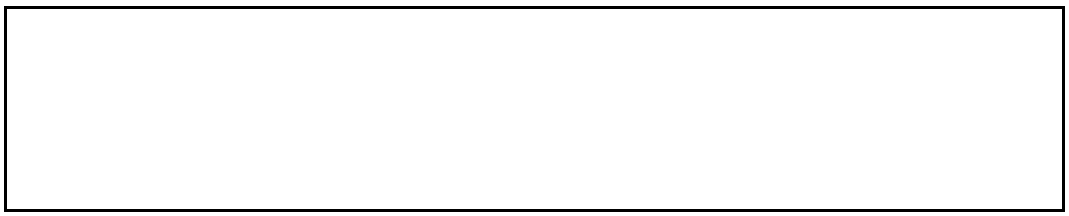


The basic ionic liquid 1-Butyl-3-methylimidazolium hydroxide, [bmim]OH, efficiently

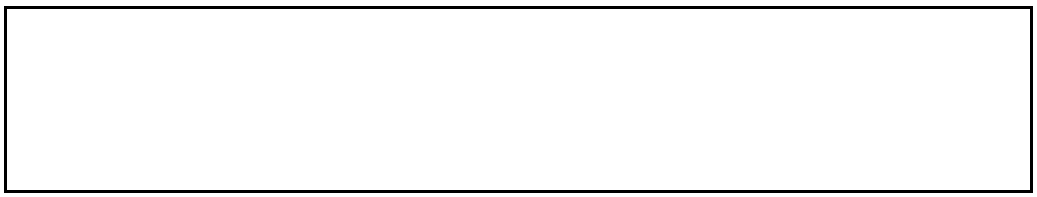
catalyzes the Knoevenagel condensation of various aliphatic and aromatic aldehydes and ketones with active methylenes at room temperature without requirement of any organic solvent.[190]



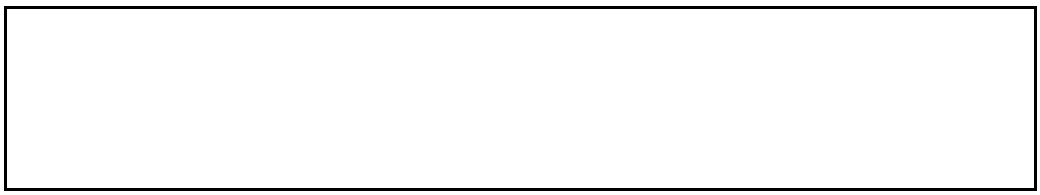
A facile, convenient, efficient and high yielding synthesis of a combinatorial library of 3-aroylcoumarins has been developed by the condensation of easily available aroylketene dithioacetals and 2-Hydroxybenzaldehydes in the presence of catalytic amount of piperidine in THF reflux.[191]



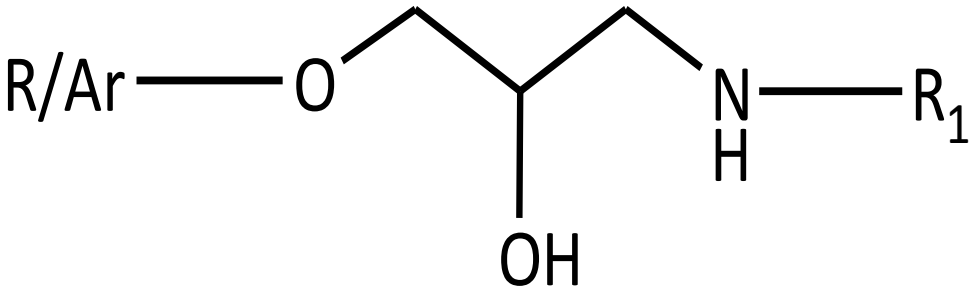
The ionic liquid 1-Butyl-3-methylimidazonium tetrafluoroborate [bmim]$BF_4$ was used for Ethylenediammonium diacetate (EDDA)-catalyzed Knoevenagel condensation between aldehydes or ketones with active methylene compounds. Catalyst and solvent were recyclable.[192]



A new carbamoyl Baker-Venkataraman rearrangement allows a general synthesis of substituted 4-Hydroxycoumarins in good overall yields. Intermediate arylketones are efficiently prepared via a Directed *ortho* Metalation - Negishi cross coupling protocol from arylcarbamates. The overall sequence provides a regiospecific anionic Friedel-Crafts complement for the construction of *ortho*-acyl phenols and coumarins.[193]



An Efficient and Practical Procedure for the Synthesis of 4-Substituted Coumarins.[194]




# MATERIALS AND METHODS

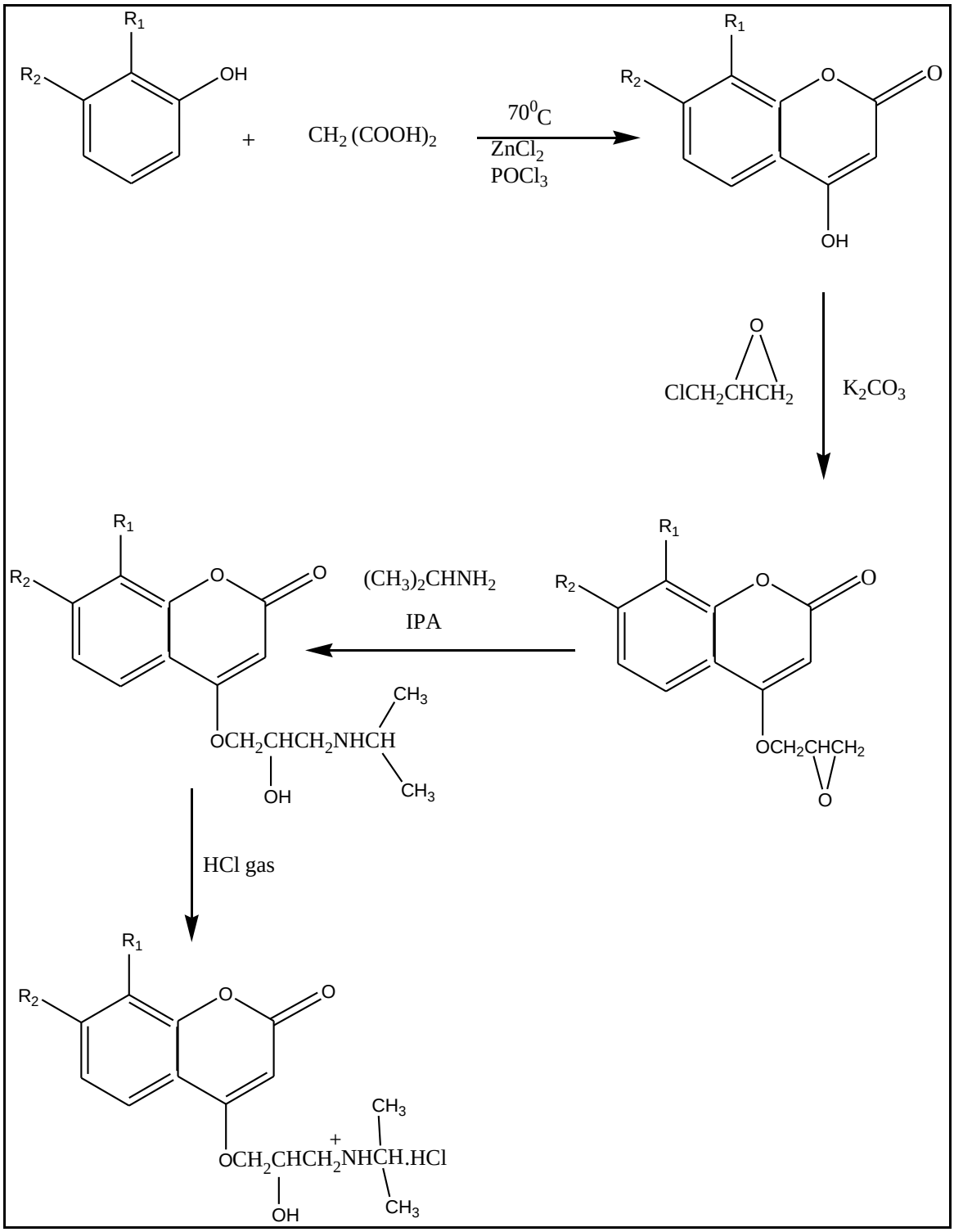
# 5. MATERIALS AND METHODS

All chemicals (Merck UK Pvt. Ltd.) were purchased without any external financial support. All materials were stored and utilized for reaction in laboratory of R. K. College of Pharmacy, Rajkot.

Synthesis of intermediates and final products, with high yield in minimum steps, was a major concern in this work. Keeping this view in mind, we optimized each step during this work. The modifications were made on trial and error basis, applying appropriate logic. We have synthesized derivatives of substituted 4-hydroxy coumarin nucleus.

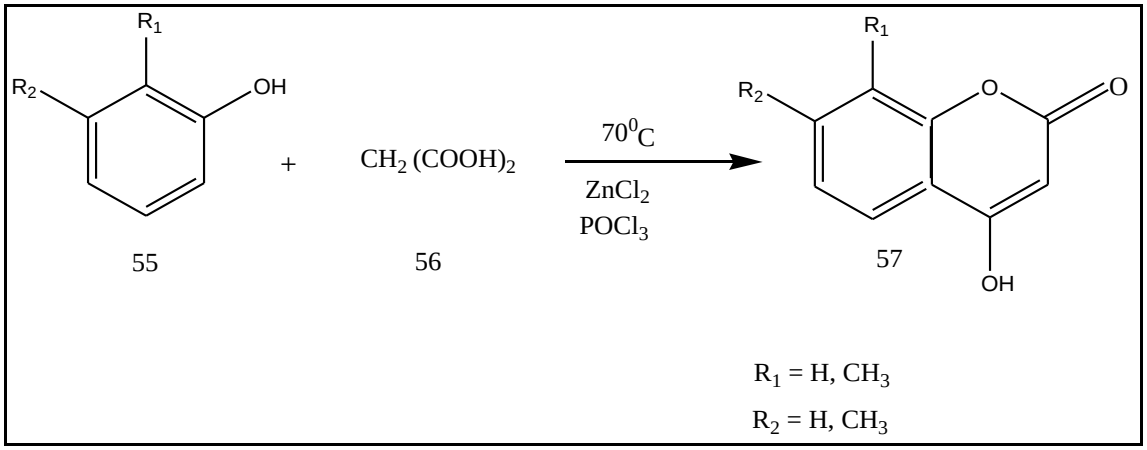
Our target was to synthesize propranolol like molecules, containing coumarin skeleton. Hence, in the first step, we synthesized 4-hydroxycoumarin from phenol which was then, converted to epoxy derivative in second step. The epoxy derivative was reacted with an amine to form alkylaminohydroxypropoxy coumarin derivative that was finally converted to its hydrochloride salt. Following synthetic scheme was used for reference -

**Synthetic Scheme**



### 5.1.1 Experimental Procedure

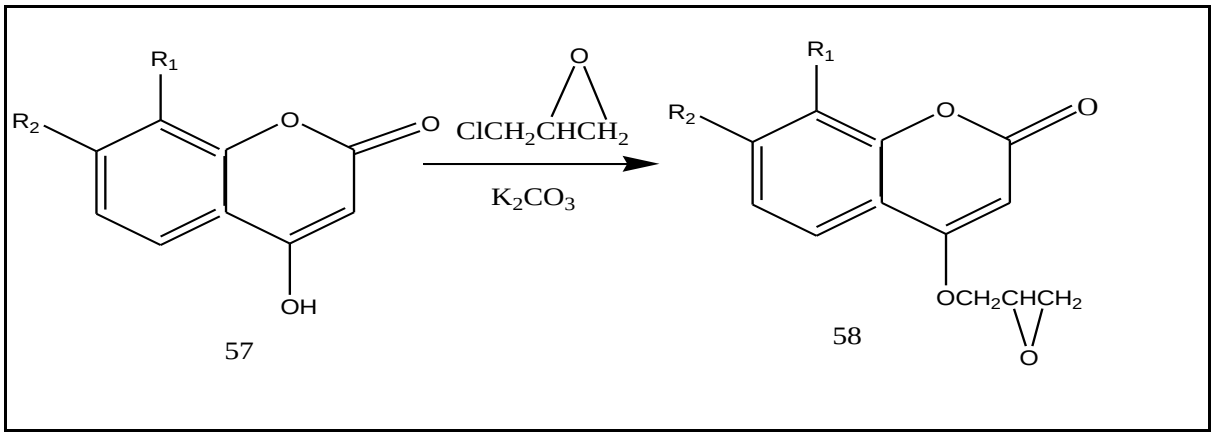
#### 5.1.1.1 Preparation of 4-hydroxy Coumarin Nucleus




The coumarin nucleus was prepared according to A. K. Shah and co-worker's method. Phenol (0.1M, 55) and malonic acid (0.1M, 56) were added to a mixture of phosphorous oxychloride (40ml) and anhydrous zinc chloride (30g) that was preheated to $60^0$C. The reaction mixture was heated for 12-15 hours at 70 $^0$C on water bath. After completion of reaction, the reaction mixture was decomposed by ice and water to form a yellow solid. This was filtered and washed with water and further triturated with 10% w/v sodium carbonate and filtered. The filtrate was cooled and slowly acidified with dilute hydrochloric acid. At the neutral point, solid product was precipitated, filtered and washed with water followed by drying. The product was recrystallized, using dilute ethanol. Reaction yield was 60 % with melting point 258-260$^0$C and Rf* value of 0.31.

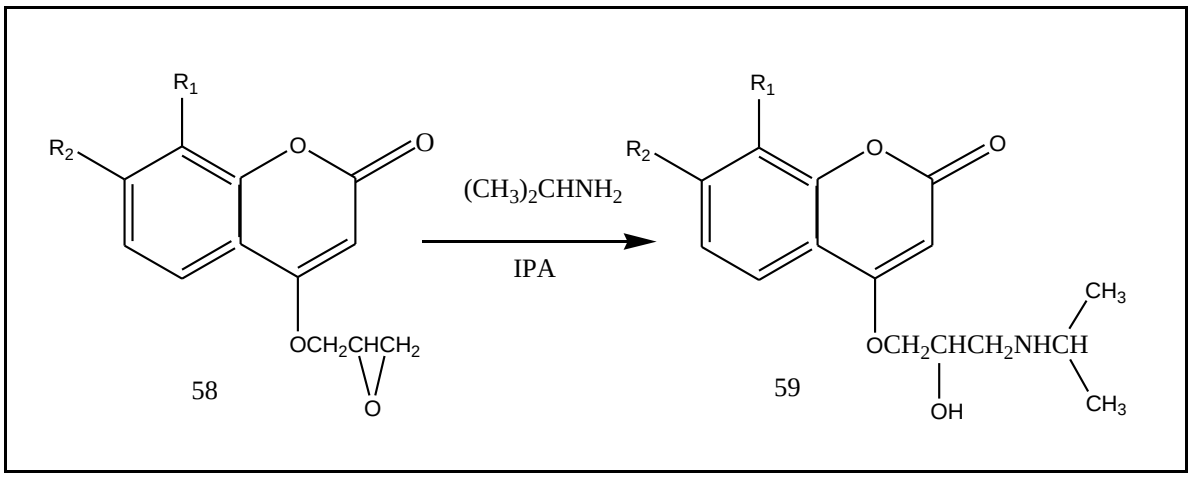
*Mobile phase: n-Hexane-Ethyl acetate 1:1

### 5.1.1.2 Preparation of 4-(2, 3-epoxypropoxy) Coumarin




In 100 ml RBF, 4-hydroxy coumarin (0.01M, 57), potassium carbonate (0.02M) and epichlorhydrin (15ml) were heated, for 14-18 hours on oil bath. The reaction mixture was filtered to remove potassium salts. Filtrate was distilled out. Approximately 7ml distillate was collected followed by addition of 25 ml toluene which on distillation gave yellow semisolid mass. The product was used for further reaction, without isolation.

### 5.1.1.3 Preparation of 4-(2-hydroxy-3-isopropylaminopropoxy) Coumarin

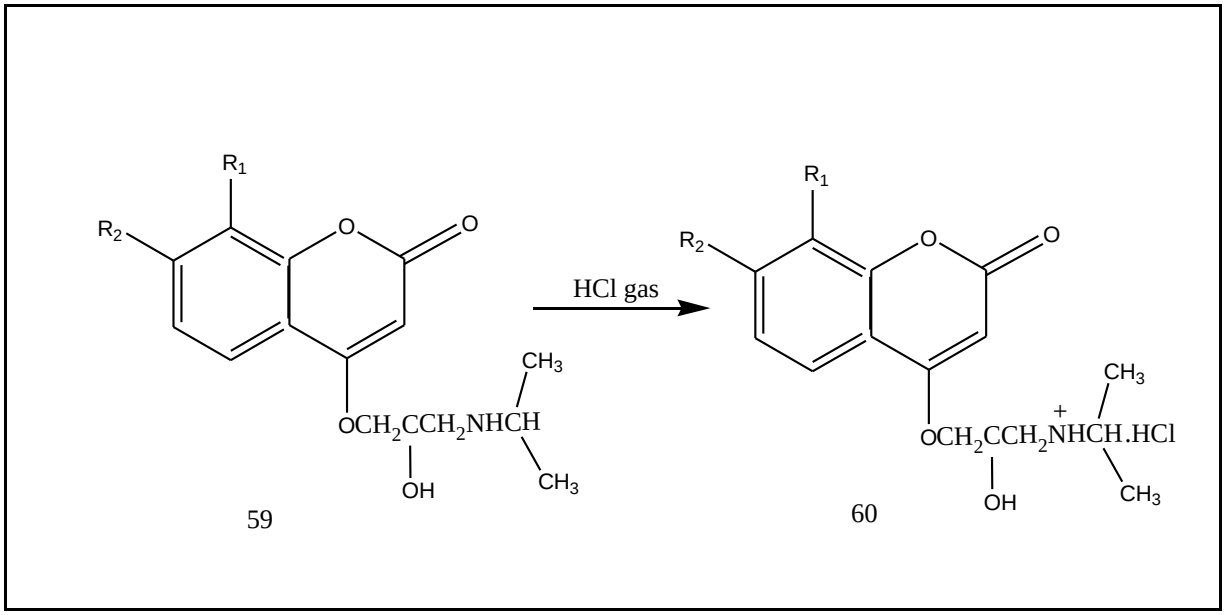



A mixture of 4-(2, 3-epoxypropoxy) coumarin (0.01 M, 58) and 25ml isopropyl alcohol was warmed in 100ml RBF to form solution. 5ml Isopropyl amine was added to the reaction mixture and refluxed for 12-15 hours, on a water bath. Under

reduced pressure, excess isopropylamine and isopropanol were distilled out to produce reddish brown, semisolid mass. This semisolid mass was used for the preparation of its hydrochloride salt.

#### 5.1.1.4 Preparation of 4-(2-hydroxy-3-isopropylaminopropoxy) Coumarin hydrochloride

The base (59) was dissolved in mixture of isopropylalcohol and ether (1:5). This solution was chilled at 0-1 $^0$C and dry HCl gas was passed in to the solution under stirring. Off- white solid was precipitated from the reaction mixture. Yield of reaction was 70% and melting point of synthesized product was 220-222 $^0$C.




59 60

## 5.2 PHARMACOLOGICAL SCREENING

### 5.2.1 Anticoagulant Activity

#### 5.2.1.1 Blood sample collection and blood analysis

For screening of anticoagulant activity, at the end of three weeks treatment, blood samples were collected from retro orbital plexuses under light ether anesthesia. The samples were collected in EDTA tube to prevent clot formation, at room temperature. Determination of clotting time was done by using Lee and White method.

#### 5.2.1.2 Apparatus

1. Sterile disposable pricking needle

2. Stop watch

3. Dry glass capillary tube (narrow diameter 1 top 2 mm, minimum 10 cm long.)

4. Cotton Swab of absorbent cotton

5. Spirit wetted cotton swab

#### 5.2.1.3 Chemicals

70 % v/v ethyl alcohol or 70 % v/v denatured spirit.

#### 5.2.1.4 Stepwise procedure

1. Blood was collected from retro orbital plexus under light anesthetized condition.

2. Stop watch was started, immediately.

3. One end of capillary was dipped into blood drop, without applying pressure.

5. At every 30 second, a small piece of capillary was broken. Stop watch was used to measure the time.

6. This process was repeated until fibrin thread appeared, at the broken end of the capillary tube.

7. The time interval was recorded between, pricking the finger and first appearance of the fibrin thread. This was noted as the clotting time of blood.

**5.2.1.5 Statistical Analysis**

Results are presented as mean ± SEM. Statistical differences between the means of the various groups were evaluated using one-way analysis of variance (ANOVA) followed by Tukey's test. Data were considered statistically significant at $P \leq 0.05$ and highly significant at $P \leq 0.001$. Statistical analysis was performed using Sigma stat statistical software.

**5.2.2 Anti Hypertensive Activity**

**5.2.2.1 Apparatus**

1. BIOPAC
2. Blood pressure coupler
3. Pair of scissors
4. Burette

**5.2.2.2 Chemicals**

1. Noradrenaline
2. Saline
3. Ketamine
4. Diazepam

**5.2.2.3 Stepwise Procedure**

1. Healthy Wistar male rats (230-250 gm) were anaesthetized with ketamine solution.

2. A midline incision was made on the skin of neck, starting from the lower end of larynx up to the upper end of thorax. Muscles were separated along the midline with

the help of pair of scissors by introducing the closed tips. The jugular vein was exposed, just under the skin at the side of neck, taking care, not to damage it.

3. The venous cannula was inserted into the vein in the same fashion, as into the artery, except that the bulldog clamp was first applied proximally and a ligature was tied a little distally while, the vein was full of blood.

4. After the cannula was tied in position, it was connected to burette filled with saline.

5. Drugs were injected through the rubber tubing near to the cannula and a constant volume of saline was allowed to run, each time after injection.

6. Trachea was exposed by retracting the pretracheal muscles, and a transverse cut was made in between two rings. A tracheal cannula was introduced into the gap between the two rings pointing toward the lung and held firmly in position with the help of ligature. The purpose of cannulating the trachea, was to allow free breathing, without any obstruction by secretions that can be cleared as and when necessary and also to provide artificial respiration, when needed and also to facilitate recording of the effects of drugs on respiration.

7. Carotid arteries that lie close to the trachea on either side along with the vein and the nerves could be easily recognized by their elastic and pulsating nature. One of these arteries was cleaned from the accompanying structure, to a sufficient length, with the help of a blunt dissector. It was then, tied near the head end, as possible. A bulldog clamp was placed, about 3 cm nearer to the head and thread was passed around the artery.

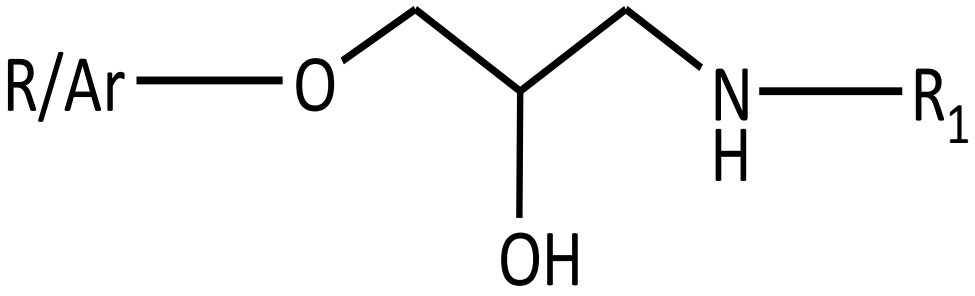
8. A cut was made carefully on the artery, close to the ligature, with the help of a sharp curved scissors so as to make a small opening through which, an arterial cannula already filled with some coagulant fluid, was inserted directing towards the heart, and firmly secured with the ligature, already in position.

9. Arterial cannula was connected to the three-way tap in manometer. The space between the manometer and the arterial cannula was filled with sodium citrate. Care was taken to avoid entry of any air bubble.

10. When the whole system was ready, and rendered free of air bubbles, the pinchcock was closed. The pressure in the manometer was then, increased to about 150 mmHg and then three way knob was turned so that the manometer was in communication with the cannula. The positive pressure was approximately equal to that of the blood pressure of the animal. Before the bulldog clamp was removed, 0.5 ml of heparin solution was injected in to the arterial cannula through the rubber tubing. The bulldog clamp was then taken off; the column of mercury rose or fell, slightly, until its pressure counterbalanced that of the blood. The writing points remained at a constant level, except for slight oscillation, due to the heart beats and respiratory movements. The height of the mercury column, midway between the top and the bottom of these oscillations, was taken as the mean arterial pressure.

**5.2.2.4 Statistical Analysis**

Results were presented as mean ± SEM. Statistical differences between the means of the various groups were evaluated using one-way analysis of variance (ANOVA) followed by Tukey's test. Data were considered statistically significant at $P \leq 0.05$. Statistical analysis was performed using Sigma stat statistical software.




# RESULTS

# 6. RESULT

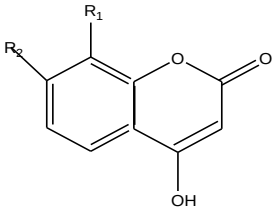
In the present project, we synthesized 30 drug molecules, with antihypertensive activity of propranolol and anticoagulant activity of Warfarin. To achieve this, we attached alkylaminohydroxypropoxy side chain to 4-hydroxy coumarin nucleus.

## 6.1 PHYSICAL DATA

The syntheses involved following steps:

1. Preparation of 4-hydroxy coumarin nucleus
2. Preparation of 4-(2, 3-epoxypropoxy) coumarin
3. Preparation of 4-(2-hydroxy-3-alkylaminopropoxy) coumarin
4. Preparation of 4-(2-hydroxy-3-alkylaminopropoxy) coumarin hydrochloride

### 6.1.1 Physical Data of Substituted 4-Hydroxycoumarin




The completion of reaction was checked by TLC (n-Hexane-Ethyl acetate 1:1). 4-hydroxy coumarin was freely soluble in methanol and chloroform and yellowish in color.

**Table 3: Physical Data of Synthesized Coumarin Nucleus**

| Sr. No | Code | $R_1$ | $R_2$ | M.F. | M. P. ($^0$C) | $R_f$ *value | Color | % Yield |
|---|---|---|---|---|---|---|---|---|
| 1 | BST 11 | H | H | $C_9H_6O_3$ | 258-260 | 0.31 | Yellow | 60 |
| 2 | BST 21 | $-CH_3$ | H | $C_{10}H_8O_3$ | 271-273 | 0.26 | Yellow | 54 |
| 3 | BST 31 | H | $-CH_3$ | $C_{10}H_8O_3$ | 278-280 | 0.28 | Yellow | 57 |

*Mobile phase: n-Hexane-Ethyl acetate 1:1

### 6.1.2 FTIR Spectra of Synthesized Coumarin Nucleus

**BST $_{11}$**

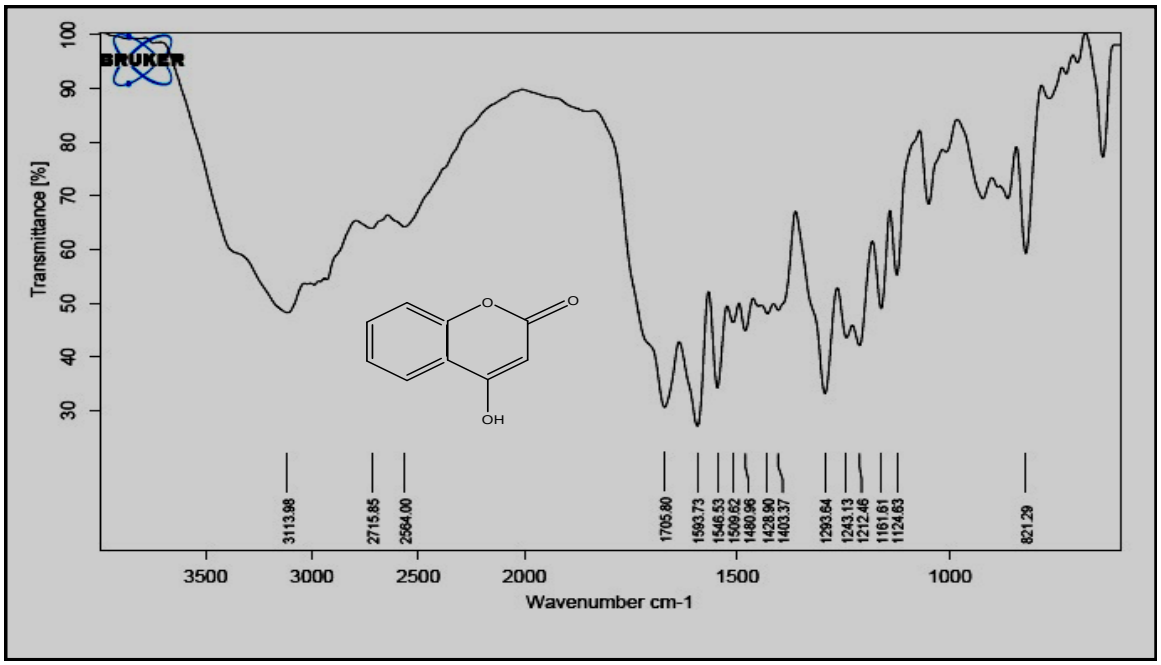



**Fig. 2 FTIR Spectra of BST $_{11}$**

**Table: 4 FTIR Spectral Data of BST $_{11}$**

| Sr. No. | Wave number ($Cm^{-1}$) | Remarks |
|---|---|---|
| 1 | 1705 | -C=O (S) |
| 2 | 1161 | -C-O (S) |
| 3 | 3113 | -O-H (S) |
| 4 | 1593 | -C=C (S) |

**BST $_{31}$**

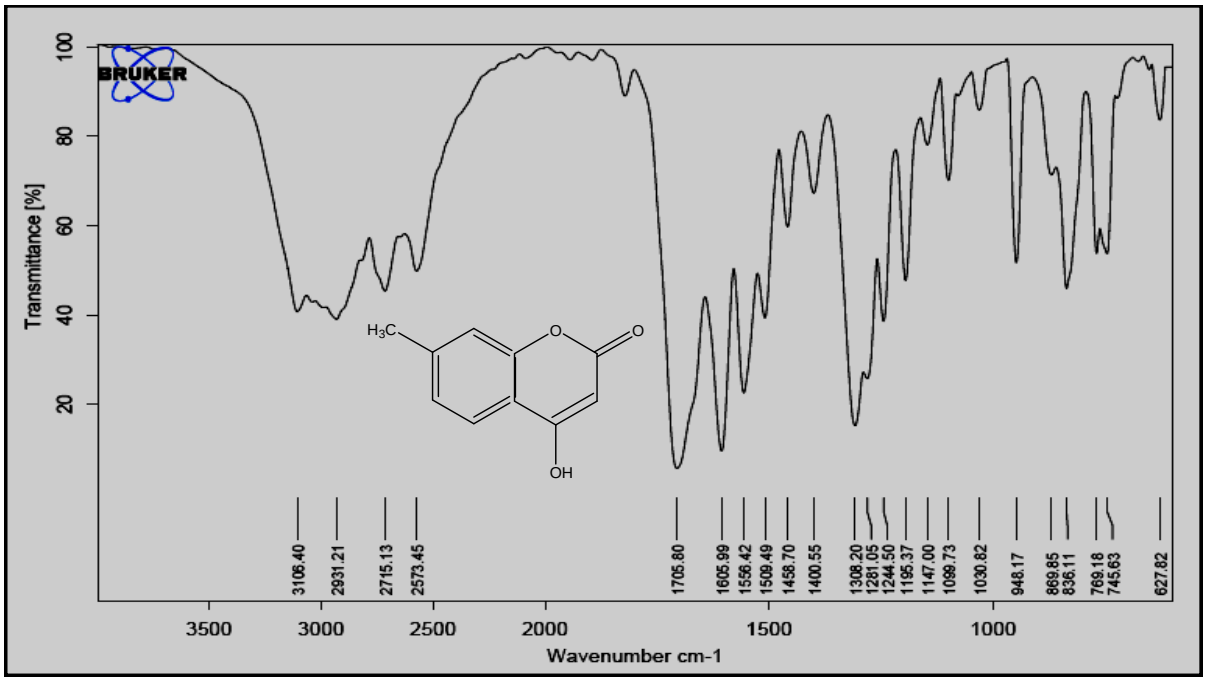



**Fig. 3 FTIR Spectra of BST $_{31}$**

**Table 5: FTIR Spectral Data of BST $_{31}$**

| Sr. No. | Wave number ($Cm^{-1}$) | Remarks |
|---|---|---|
| 1 | 1705 | -C=O (S) |
| 2 | 1147 | -C-O (S) |
| 3 | 3106 | -O-H (S) |
| 4 | 1556 | -C=C (S) |
| 5 | 2981 | -C-H (Ar) (S) |

### 6.1.3 Physical Data of 4-(2-hydroxy-3-isopropylaminopropoxy) Coumarin hydrochloride

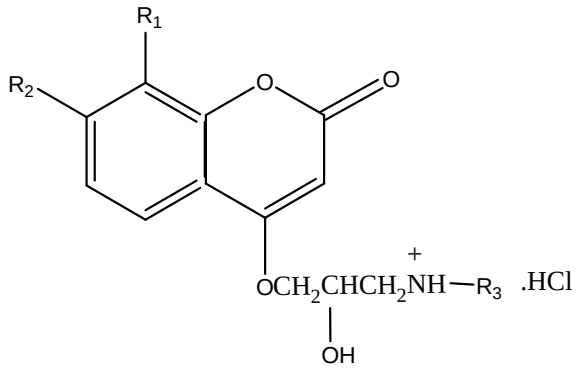



**Table 6: Physical Data of 4-(2-hydroxy-3-isopropylaminopropoxy) coumarin hydrochloride**

| Sr. No | Code | $R_1$ | $R_2$ | $R_3$ | M.F. | M. P.(°C) | $R_f$ * value | %Yield |
|---|---|---|---|---|---|---|---|---|
| 1 | BLT 1 | H | H | $-CH(CH_3)_2$ | $C_{15}H_{20}NO_4Cl$ | 220-222 | 0.37 | 60 |
| 2 | BLT 2 | H | H | $-C(CH_3)_3$ | $C_{16}H_{22}NO_4Cl$ | 212-214 | 0.35 | 68 |
| 3 | BLT 3 | H | H | $-CH_2(CH_2)_2CH_3$ | $C_{16}H_{22}NO_4Cl$ | 218-220 | 0.33 | 62 |
| 4 | BLT 4 | H | H | $-CH_2(CH_2)_3CH_3$ | $C_{17}H_{24}NO_4Cl$ | 228-230 | 0.33 | 58 |
| 5 | BLT 5 | H | H | $-CH(CH_2)_2$ | $C_{15}H_{18}NO_4Cl$ | 222-224 | 0.33 | 54 |
| 6 | BLT 6 | H | H | $-CH(C_2H_2)_5$ | $C_{18}H_{24}NO_4Cl$ | 230-232 | 0.28 | 54 |
| 7 | BLT 7 | H | H | $-(C_2H_5)_2$ | $C_{16}H_{22}NO_4Cl$ | 226-228 | 0.35 | 62 |
| 8 | BLT 8 | H | H | $-(C_2H_40H)_2$ | $C_{16}H_{22}NO_6Cl$ | 218-220 | 0.39 | 61 |
| 9 | BLT 9 | H | H | $-(C_4H_9)_2$ | $C_{20}H_{30}NO_4Cl$ | 225-227 | 0.28 | 54 |
| 10 | BLT 10 | H | H | $-(CH_3)_2$ | $C_{14}H_{18}NO_4Cl$ | 220-222 | 0.35 | 52 |
| 11 | BLT 11 | $-CH_3$ | H | $-CH(CH_3)_2$ | $C_{16}H_{22}NO_4Cl$ | 245-247 | 0.31 | 56 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 12 | BLT 12 | $-CH_3$ | H | $-C(CH_3)_3$ | $C_{17}H_{24}NO_4Cl$ | 240-242 | 0.30 | 59 |
| 13 | BLT 13 | $-CH_3$ | H | $-CH_2(CH_2)_2CH_3$ | $C_{17}H_{24}NO_4Cl$ | 247-249 | 0.25 | 61 |
| 14 | BLT 14 | $-CH_3$ | H | $-CH_2(CH_2)_3CH_3$ | $C_{18}H_{26}NO_4Cl$ | 259-261 | 0.27 | 53 |
| 15 | BLT 15 | $-CH_3$ | H | $-CH(CH_2)_2$ | $C_{16}H_{20}NO_4Cl$ | 251-253 | 0.31 | 55 |
| 16 | BLT 16 | $-CH_3$ | H | $-CH(C_2H_2)_5$ | $C_{19}H_{26}NO_4Cl$ | 261-262 | 0.25 | 48 |
| 17 | BLT 17 | $-CH_3$ | H | $-(C_2H_5)_2$ | $C_{17}H_{24}NO_4Cl$ | 270-272 | 0.31 | 50 |
| 18 | BLT 18 | $-CH_3$ | H | $-(C_2H_40H)_2$ | $C_{17}H_{24}NO_6Cl$ | 258-260 | 0.40 | 38 |
| 19 | BLT 19 | $-CH_3$ | H | $-(C_4H_9)_2$ | $C_{21}H_{32}NO_4Cl$ | 270-272 | 0.25 | 47 |
| 20 | BLT 20 | $-CH_3$ | H | $-(CH_3)_2$ | $C_{15}H_{20}NO_4Cl$ | 262-264 | 0.31 | 38 |
| 21 | BLT 21 | H | $-CH_3$ | $-CH(CH_3)_2$ | $C_{16}H_{22}NO_4Cl$ | 251-253 | 0.31 | 50 |
| 22 | BLT 22 | H | $-CH_3$ | $-C(CH_3)_3$ | $C_{17}H_{24}NO_4Cl$ | 244-246 | 0.24 | 48 |
| 23 | BLT 23 | H | $-CH_3$ | $-CH_2(CH_2)_2CH_3$ | $C_{17}H_{24}NO_4Cl$ | 251-253 | 0.28 | 47 |
| 24 | BLT 24 | H | $-CH_3$ | $-CH_2(CH_2)_3CH_3$ | $C_{18}H_{26}NO_4Cl$ | 263-265 | 0.28 | 45 |
| 25 | BLT 25 | H | $-CH_3$ | $-CH(CH_2)_2$ | $C_{16}H_{20}NO_4Cl$ | 257-259 | 0.33 | 46 |
| 26 | BLT 26 | H | $-CH_3$ | $-CH(C_2H_2)_5$ | $C_{19}H_{26}NO_4Cl$ | 245-247 | 0.28 | 45 |
| 27 | BLT 27 | H | $-CH_3$ | $-(C_2H_5)_2$ | $C_{17}H_{24}NO_4Cl$ | 278-280 | 0.35 | 48 |
| 28 | BLT 28 | H | $-CH_3$ | $-(C_2H_4OH)_2$ | $C_{17}H_{24}NO_6Cl$ | 258-260 | 0.41 | 38 |
| 29 | BLT 29 | H | $-CH_3$ | $-(C_4H_9)_2$ | $C_{21}H_{32}NO_4Cl$ | 270-272 | 0.28 | 43 |
| 30 | BLT 30 | H | $-CH_3$ | $-(CH_3)_2$ | $C_{15}H_{20}NO_4Cl$ | 265-267 | 0.25 | 36 |

## 6.2 SPECTRAL DATA

### 6.2.1 IR Spectra

Synthesized compounds conformed to the spectral analysis. Infra Red Spectra were taken on **Shimadzu FT-IR-8400** Spectrometer, using KBr Pellet method. The characteristic peak of carbonyl group in coumarin moiety was observed at 1700-1722 $cm^{-1}$ frequency, while C-O streching of ring skeleton was observed at 1160-1125 $cm^{-1}$ frequency. The N-H streching of secondary amine gave a broad peak, between 3350-3300 $cm^{-1}$ frequency. The C-N streching was observed at 1250-1235 $cm^{-1}$ frequency. The –OH bending was observed at 1380-1310 $cm^{-1}$ frequency. Other frequencies, observed due to ring skeleton, were around 1600-1450 $cm^{-1}$ frequency of C=C stretching and 2900-3000 $cm^{-1}$ frequency, was due to C-H stretching

### 6.2.2 Mass Spectra

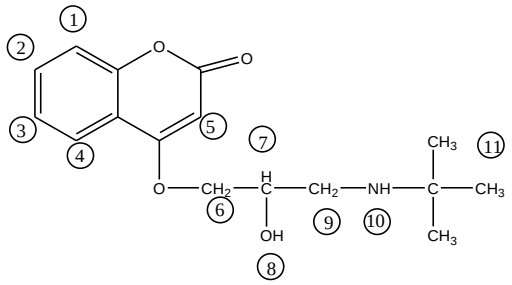
Further conformation of the molecular structure was evaluated by mass spectra. The mass spectrum of compounds were recorded by Shimadzu GC-MS-QP-2010 spectrometer. The molecular ion peak and the base peak, in all compounds, were clearly obtained in mass spectral study. The molecular ion peaks were found to be in agreement with molecular weight of the respective compounds.

### 6.2.3 $^{1}$H NMR Spectra

$^{1}$H NMR Spectra were recorded on a Bruker AC 400 MHz NMR Spectrometer using MeOD as solvent. In the NMR spectra of 4-(2-hydroxy-3-tertiarybutyl aminopropoxy) coumarin various proton values of methylene ($-CH_2$), amine (>NH), Hydroxy (R-OH) methyl ($-CH_3$) and aromatic protons (Ar-H) etc. were observed as under.

NMR Spectrum of compound $BLT_2$, was taken which showed methylene ($-CH_2$), amine (>NH), hydroxy (R-OH) methyl ($-CH_3$) and aromatic protons (Ar-H) peaks. The values for methylene ($-CH_2$) proton was observed between 2.77 and 2.86 δ ppm. Aromatic protons showed the multiplet between 7.35-8.05 δ ppm. The singlet peak of amine (-N<u>H</u>) was observed at 3.88-3.91 δ ppm. The value of methyl proton (-C<u>H</u>$_3$) was observed as singlet at 1.18 δ ppm. Multiplet were observed at 4.26-4.29 ppm of

(-$CH_2$C<u>H</u>$CH_2$) proton and triplet was obtained at 4.16-4.22 ppm due to (-$CH_2$C<u>H</u>) group.



## 6.2.4 FTIR SPECTRA OF SYNTHESIZED COMPOUNDS

**BLT $_5$**

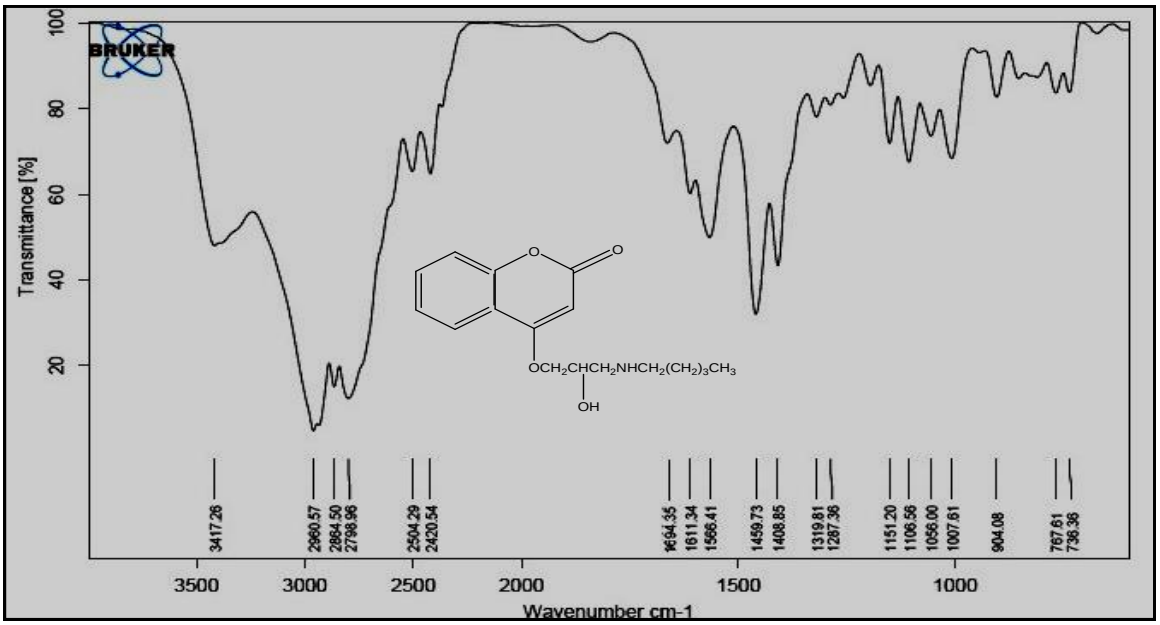



**Fig. 4 FTIR Spectra of BLT $_5$**

**Table.7 FTIR Spectral Data of BLT $_5$**

| Sr. No. | Wave number ($Cm^{-1}$) | Remarks | |
|---|---|---|---|
| 1 | 2864 | -C-H (s) | |
| 2 | 1287 | -C-N (s) | |
| 3 | 3417 | N-H (s) | —OCH$_2$CHCH$_2$NHCH$_2$(CH$_2$)$_3$CH$_3$ (OH) |
| 4 | 1319 | -OH(b) | |
| 5 | 1056 | -C-O (S) | |
| 6 | 767 | -C-O (b) | |
| 7 | 1694 | -C=O (S) | |
| 8 | 1151 | -C-O (S) | |
| 9 | 2960 | -C-H (Ar) (S) | |
| 10 | 1566 | -C=C (S) | |

**BLT 8**

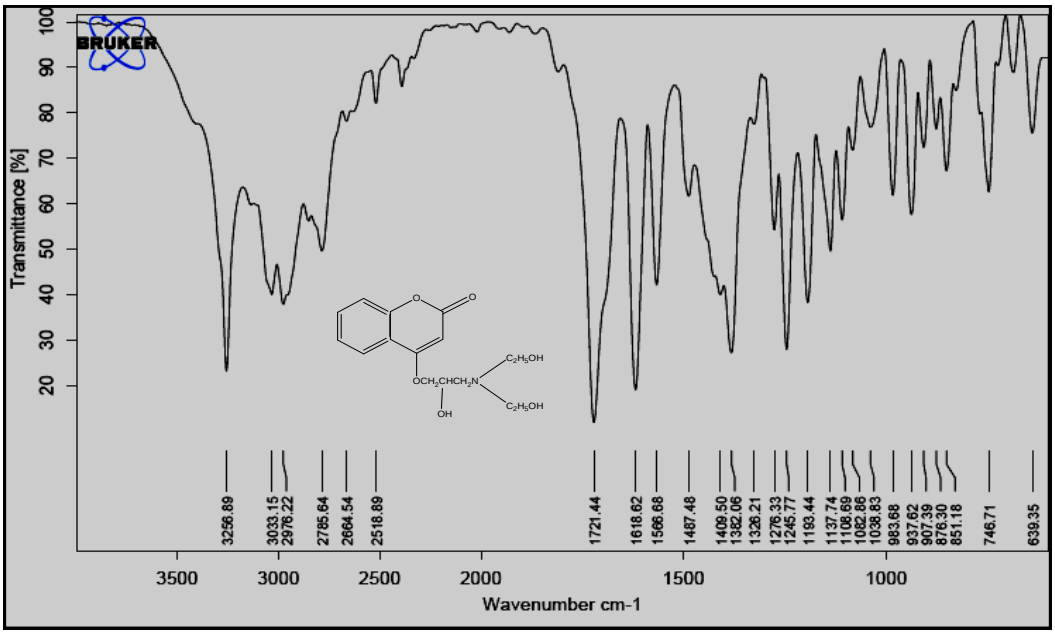
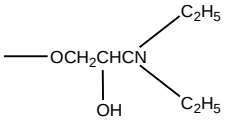
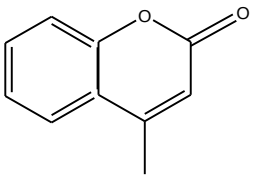



**Fig. 5 FTIR Spectra of BLT 8**

**Table 8: FTIR Spectral Data of BLT 8**

| Sr. No. | Wave number ($Cm^{-1}$) | Remarks | |
|---|---|---|---|
| 1 | 2976 | -C-H (s) |  |
| 2 | 1245 | -C-N (s) | |
| 3 | 1393 | -OH(b) | |
| 4 | 1038 | -C-O (S) | |
| 5 | 746 | -C-O (b) | |
| 6 | 1721 | -C=O (S) |  |
| 7 | 1160 | -C-O (S) | |
| 8 | 3033 | -C-H (Ar) (S) | |
| 9 | 1566 | -C=C (S) | |

**BLT $_{12}$**

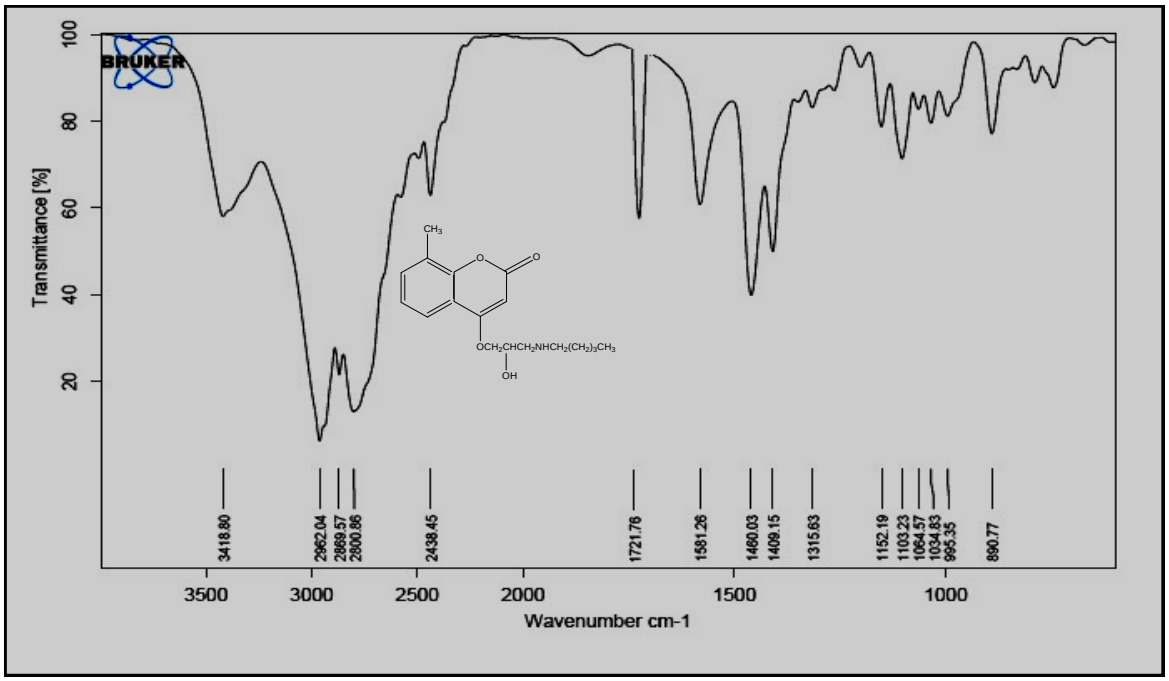



**Fig. 6 FTIR Spectra of BLT $_{12}$**

**Table 9: FTIR Spectral Data of BLT $_{12}$**

| Sr. No. | Wave number ($Cm^{-1}$) | Remarks | |
|---|---|---|---|
| 1 | 2869 | -C-H (s) | |
| 2 | 1103 | -C-N (s) | |
| 3 | 3418 | N-H (s) | |
| 4 | 1315 | -OH(b) | |
| 5 | 1034 | -C-O (S) | |
| 6 | 1721 | -C=O (S) | |
| 7 | 1152 | -C-O (S) | |
| 8 | 2962 | -C-H (Ar) (S) | |
| 9 | 1581 | -C=C (S) | |

**BLT $_{19}$**

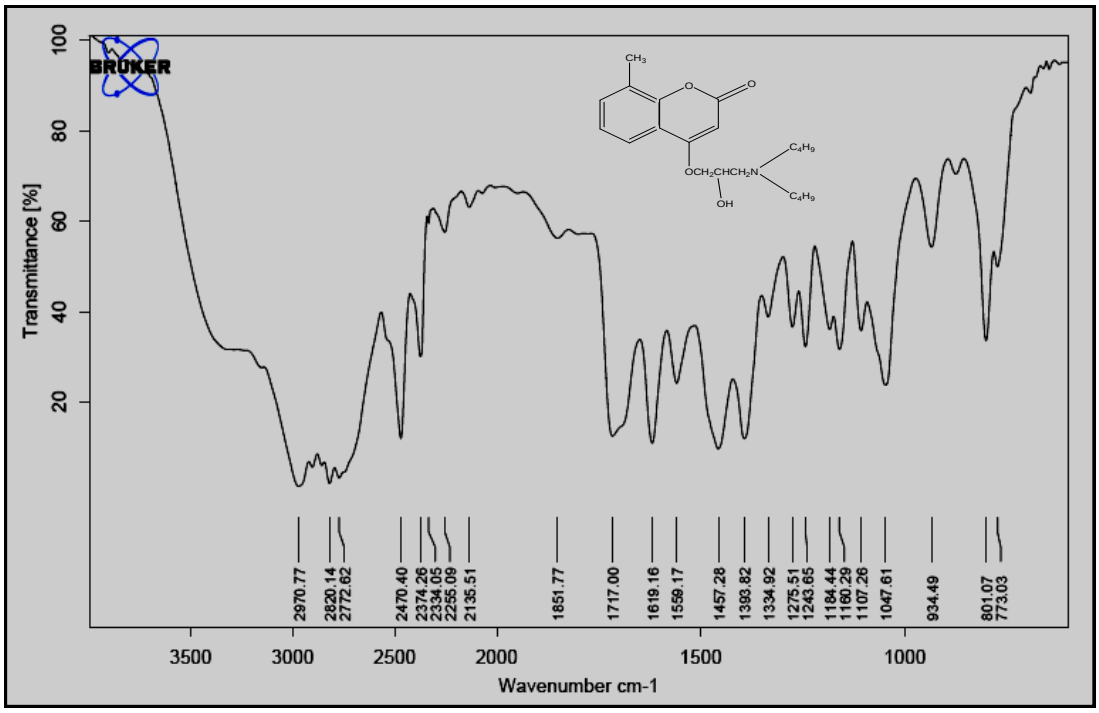
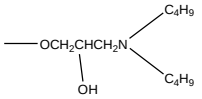
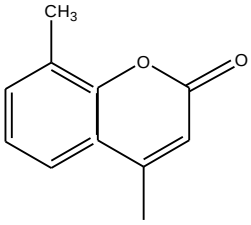



**Fig. 7 FTIR Spectra of BLT $_{19}$**

**Table 10: FTIR Spectral Data of BLT $_{19}$**

| Sr. No. | Wave number ($Cm^{-1}$) | Remarks | |
|---|---|---|---|
| 1 | 2820 | -C-H (s) | |
| 2 | 1243 | -C-N (s) | |
| 3 | 1393 | -OH(b) |  |
| 4 | 1047 | -C-O (S) | |
| 5 | 773 | -C-O (b) | |
| 6 | 1717 | -C=O (S) |  |
| 7 | 1160 | -C-O (S) | |
| 8 | 2970 | -C-H (Ar) (S) | |
| 9 | 1559 | -C=C (S) | |

**BLT $_{23}$**

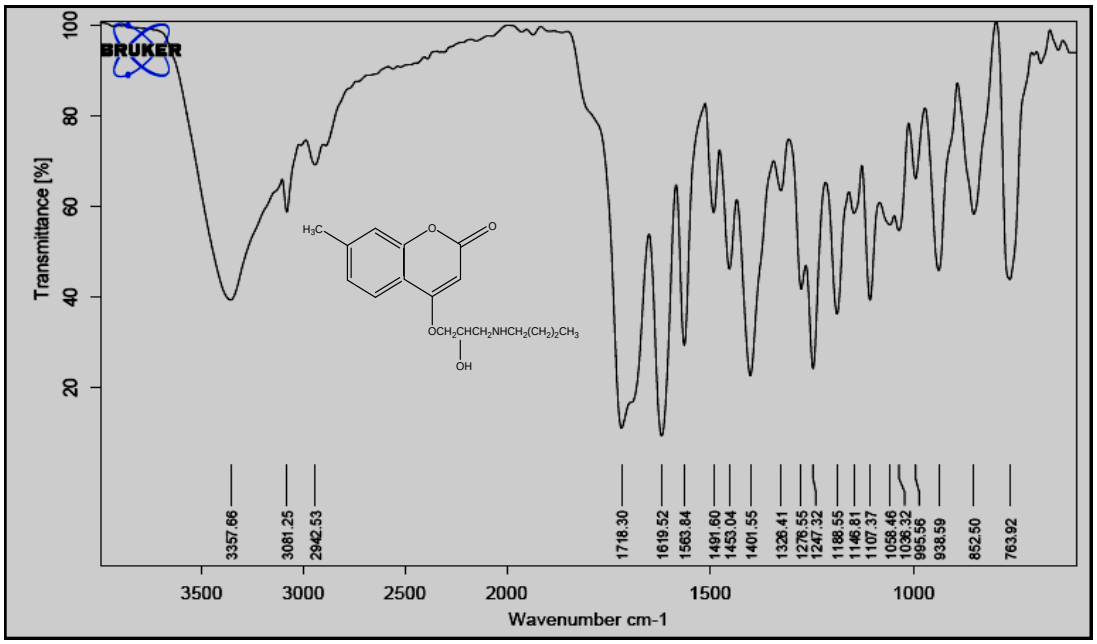



**Fig. 8 FTIR Spectra of BLT $_{23}$**

**Table 11: FTIR Spectral Data of BLT $_{23}$**

| Sr. No. | Wave number ($Cm^{-1}$) | Remarks | |
|---|---|---|---|
| 1 | 2942 | -C-H (s) | |
| 2 | 1247 | -C-N (s) | |
| 3 | 3357 | -N-H (s) | |
| 4 | 1326 | -OH(b) | |
| 5 | 1036 | -C-O (S) | |
| 6 | 763 | -C-O (b) | |
| 7 | 1718 | -C=O (S) | |
| 8 | 1146 | -C-O (S) | |
| 9 | 3081 | -C-H (Ar) (S) | |
| 10 | 1563 | -C=C (S) | |

**BLT $_{27}$**

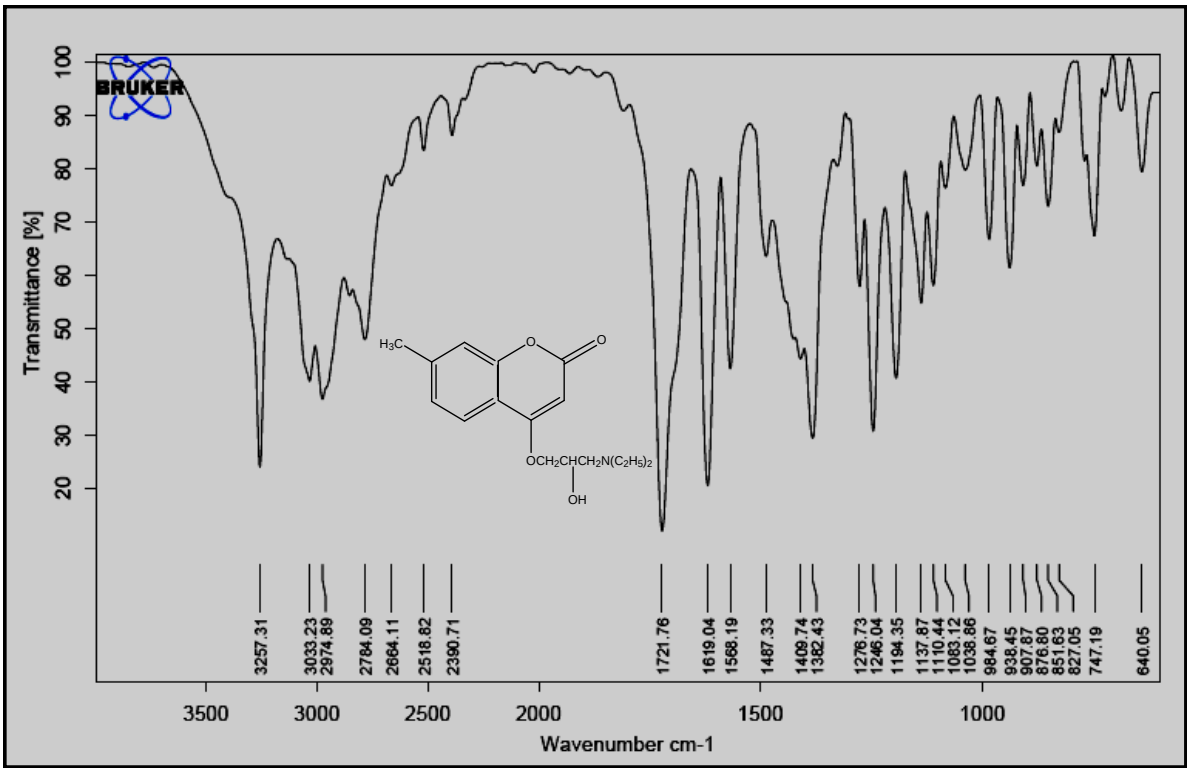



**Fig. 9 FTIR Spectra of BLT $_{27}$**

**Table 12: FTIR Spectral Data of BLT $_{27}$**

| Sr. No. | Wave number ($Cm^{-1}$) | Remarks | |
|---|---|---|---|
| 1 | 2974 | -C-H (s) | |
| 2 | 1246 | -C-N (s) | |
| 3 | 1382 | -OH(b) | |
| 4 | 1038 | -C-O (S) | |
| 5 | 747 | -C-O (b) | |
| 6 | 1721 | -C=O (S) | |
| 7 | 1137 | -C-O (S) | |
| 8 | 3033 | -C-H (Ar) (S) | |
| 9 | 1568 | -C=C (S) | |

**BLT $_{28}$**

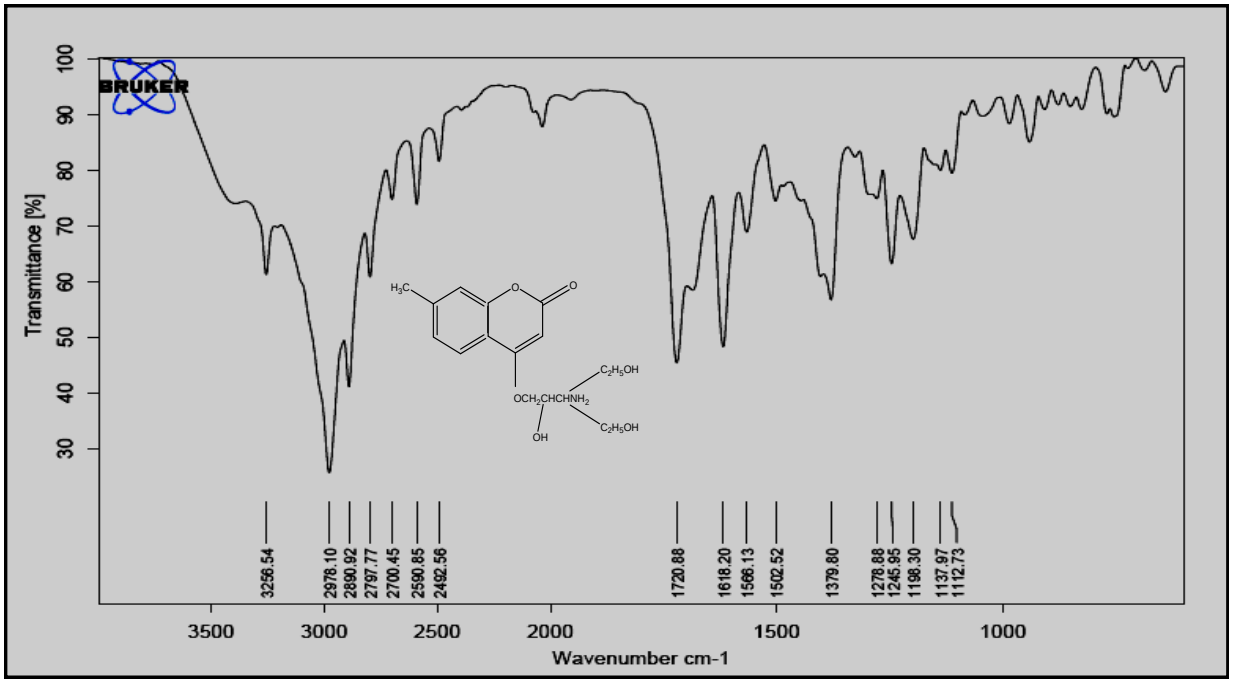
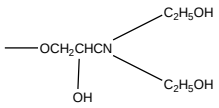
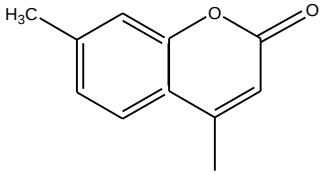



**Fig. 10 FTIR Spectra of BLT $_{28}$**

**Table 13: FTIR Spectral Data of BLT $_{28}$**

| Sr. No. | Wave number ($Cm^{-1}$) | Remark | |
|---|---|---|---|
| 1 | 2978 | -C-H (s) |  |
| 2 | 1245 | -C-N (s) | |
| 3 | 1379 | -OH(b) | |
| 6 | 1720 | -C=O (S) |  |
| 7 | 1137 | -C-O (S) | |
| 8 | 3256 | -C-H (Ar) (S) | |
| 9 | 1566 | -C=C (S) | |

## 6.2.5 MASS SPECTRA OF SYNTHESIZED COMPOUNDS

**BLT $_1$**

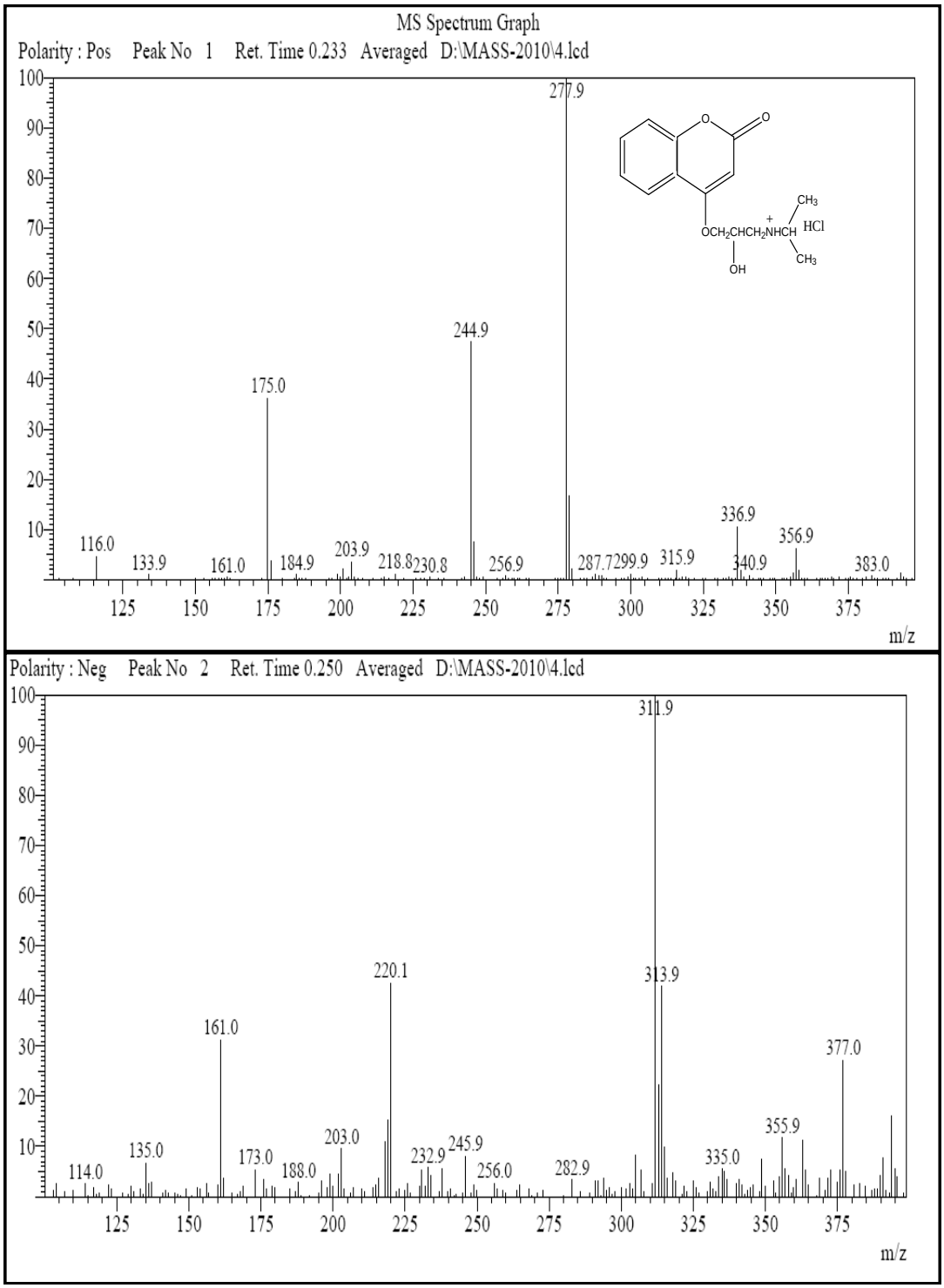
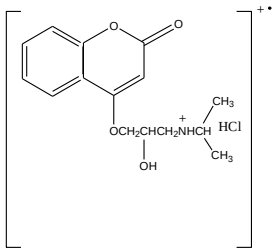
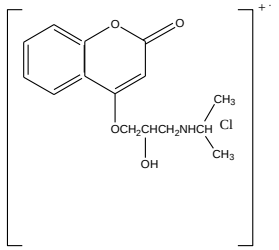
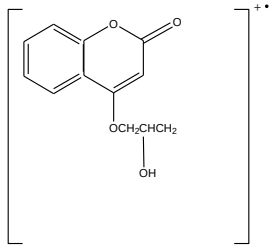
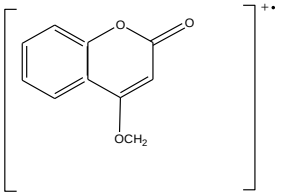
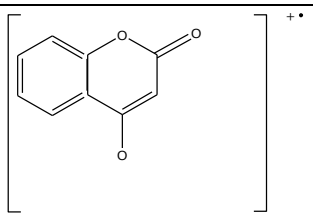



**Figure 11: Mass Spectra of BLT $_1$**

**Table 14: Mass Spectral Data of BLT $_1$**

| Sr. No. | Structure | m/e |
|---|---|---|
| 1 |  | 312.5 |
| 2 |  | 311.5 |
| 3 |  | 219 |
| 4 |  | 175.0 |
| 5 |  | 161.0 |

## BLT $_2$

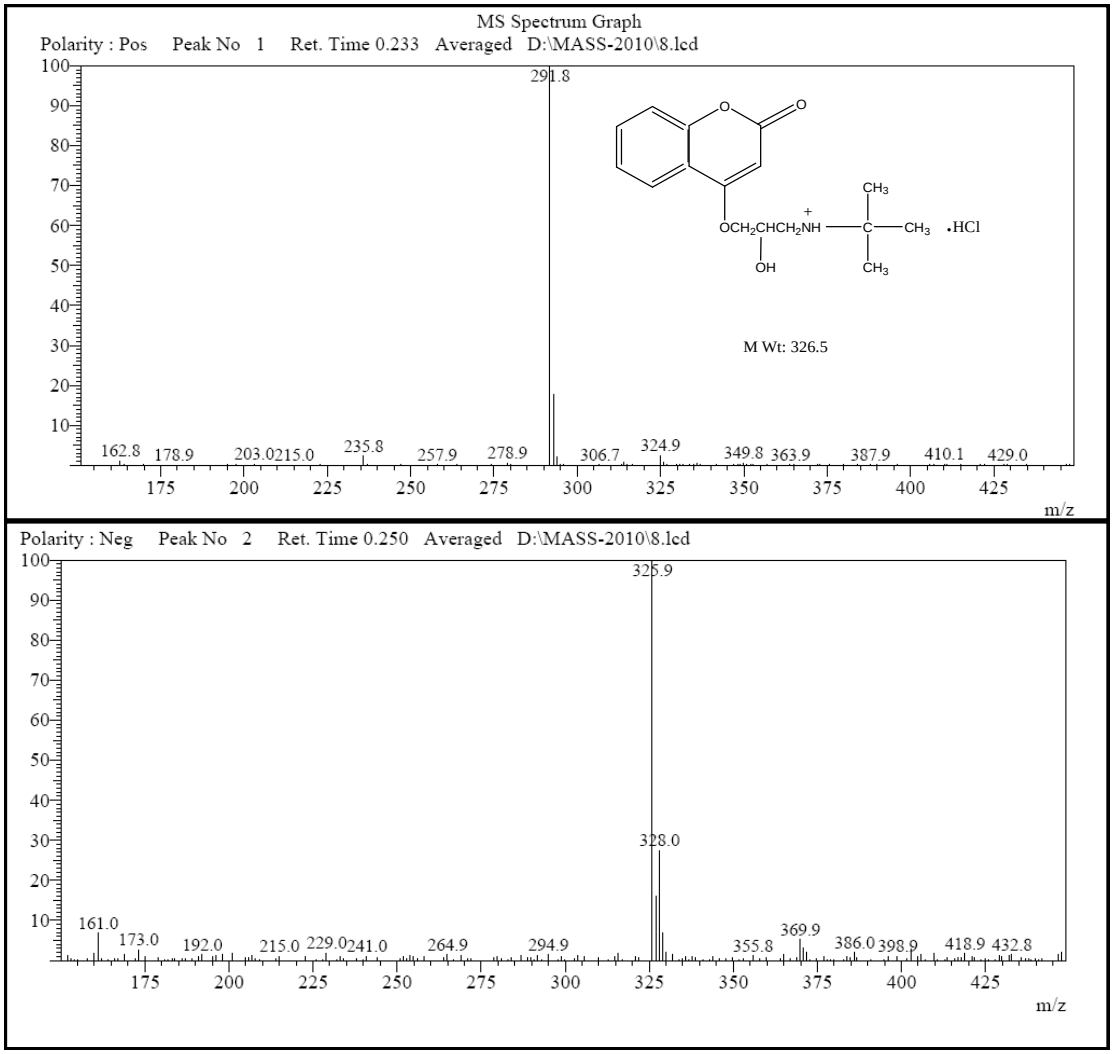
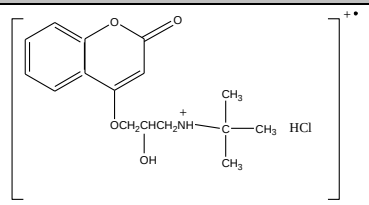
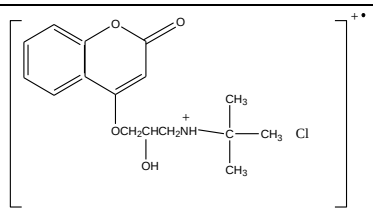
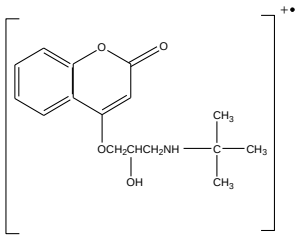
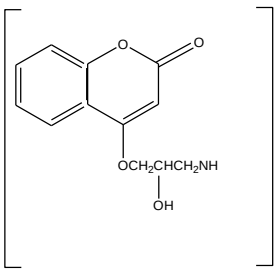
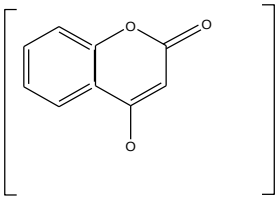



**Figure 12: Mass Spectra of BLT $_2$**

**Table 15: Mass Spectral Data of BLT $_2$**

| Sr. No. | Structure | m/e |
|---|---|---|
| 1 |  | 326.5 |
| 2 |  | 325.5 |
| 3 |  | 291 |
| 4 |  | 234 |
| 5 |  | 161.0 |

**BLT $_6$**

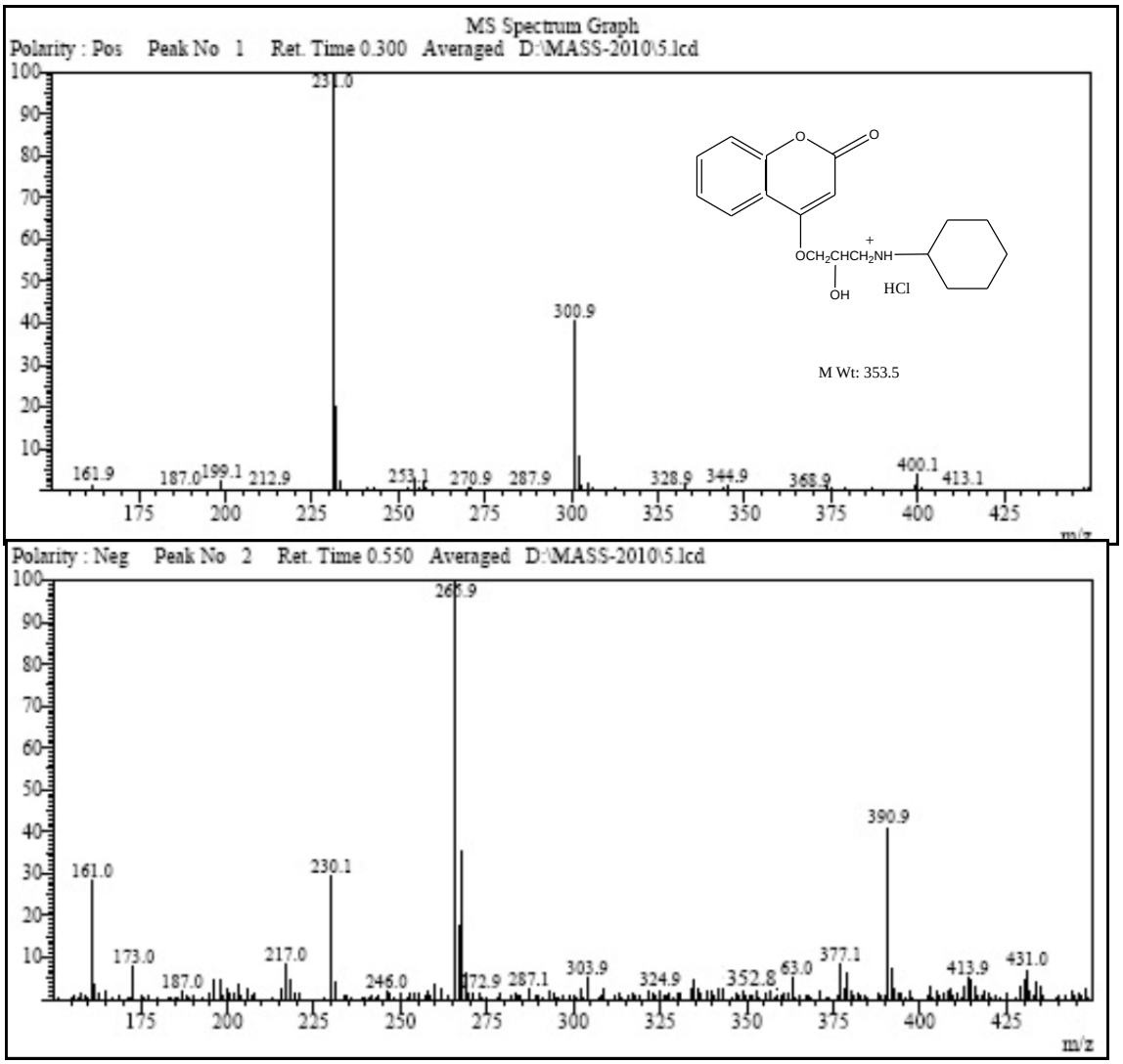
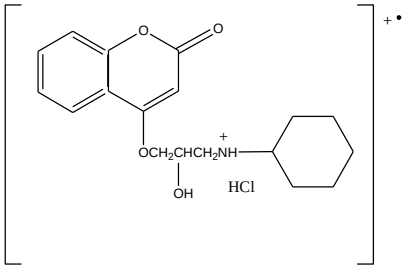
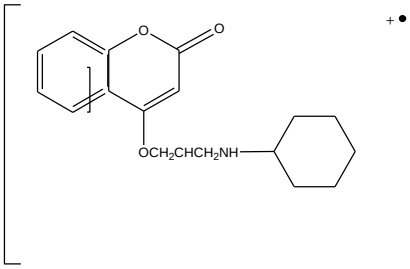
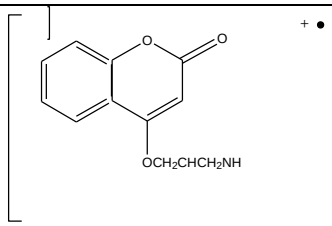
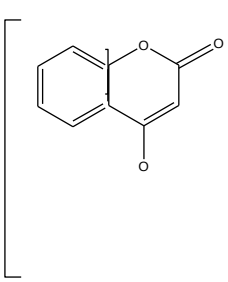



**Figure 13: Mass Spectra of BLT $_6$**

**Table 16: Mass Spectral Data of BLT $_6$**

| Sr. No. | Structure | m/e |
|---|---|---|
| 1 |  | 353.5 |
| 2 |  | 300 |
| 3 |  | 216 |
| 4 |  | 161 |

## BLT $_7$

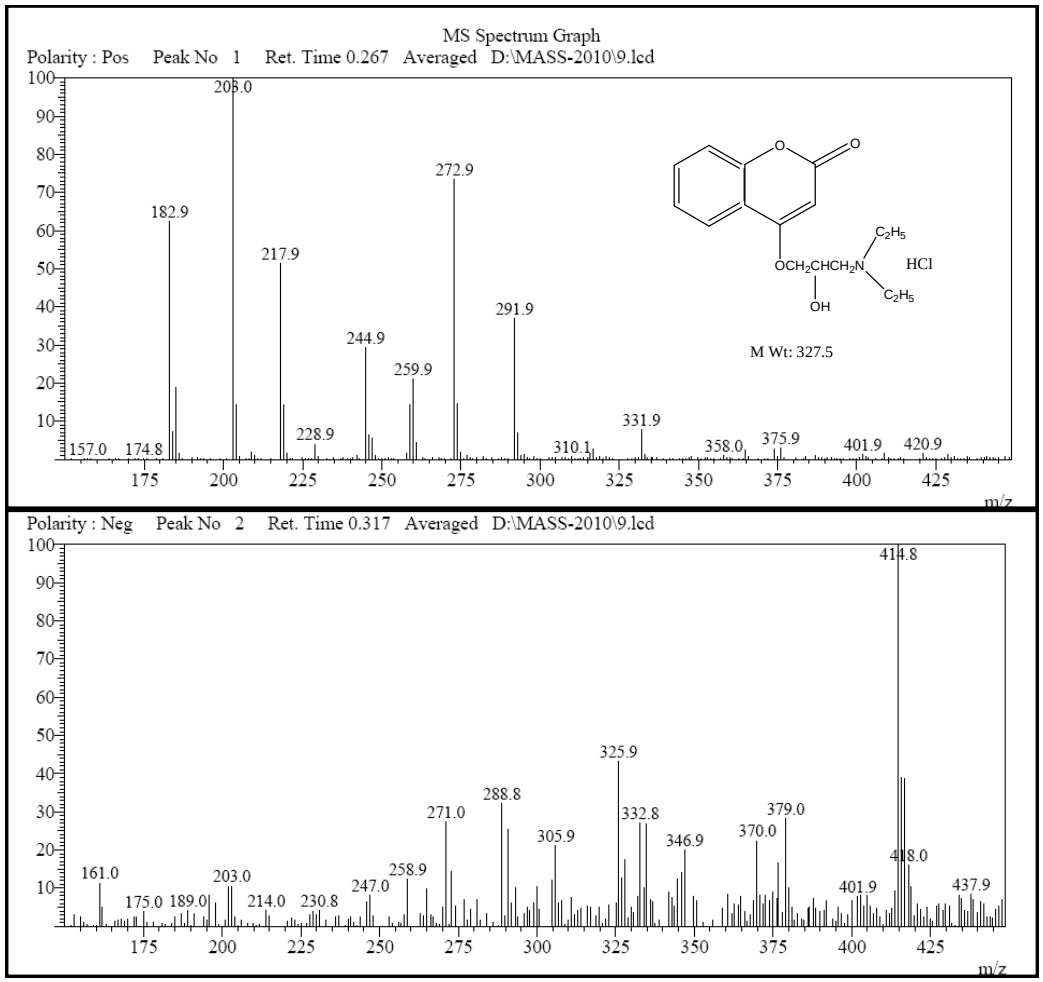
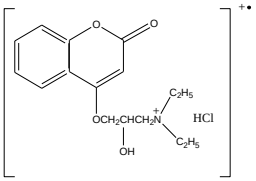
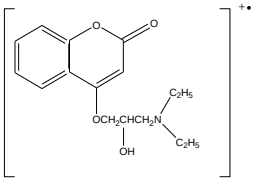
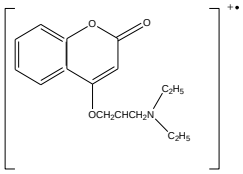
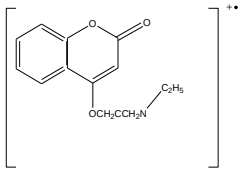
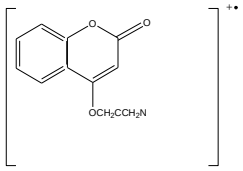
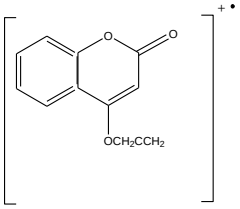
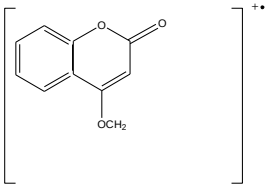



**Figure 14: Mass Spectra of BLT $_7$**

**Table 17: Mass Spectral Data of BLT $_7$**

| Sr. No. | Structure | m/e |
|---|---|---|
| 1 |  | 327.5 |
| 2 |  | 291 |
| 3 |  | 274 |
| 4 |  | 245 |
| 5 |  | 216 |
| 6 |  | 202 |
| 7 |  | 175 |

**BLT $_8$**

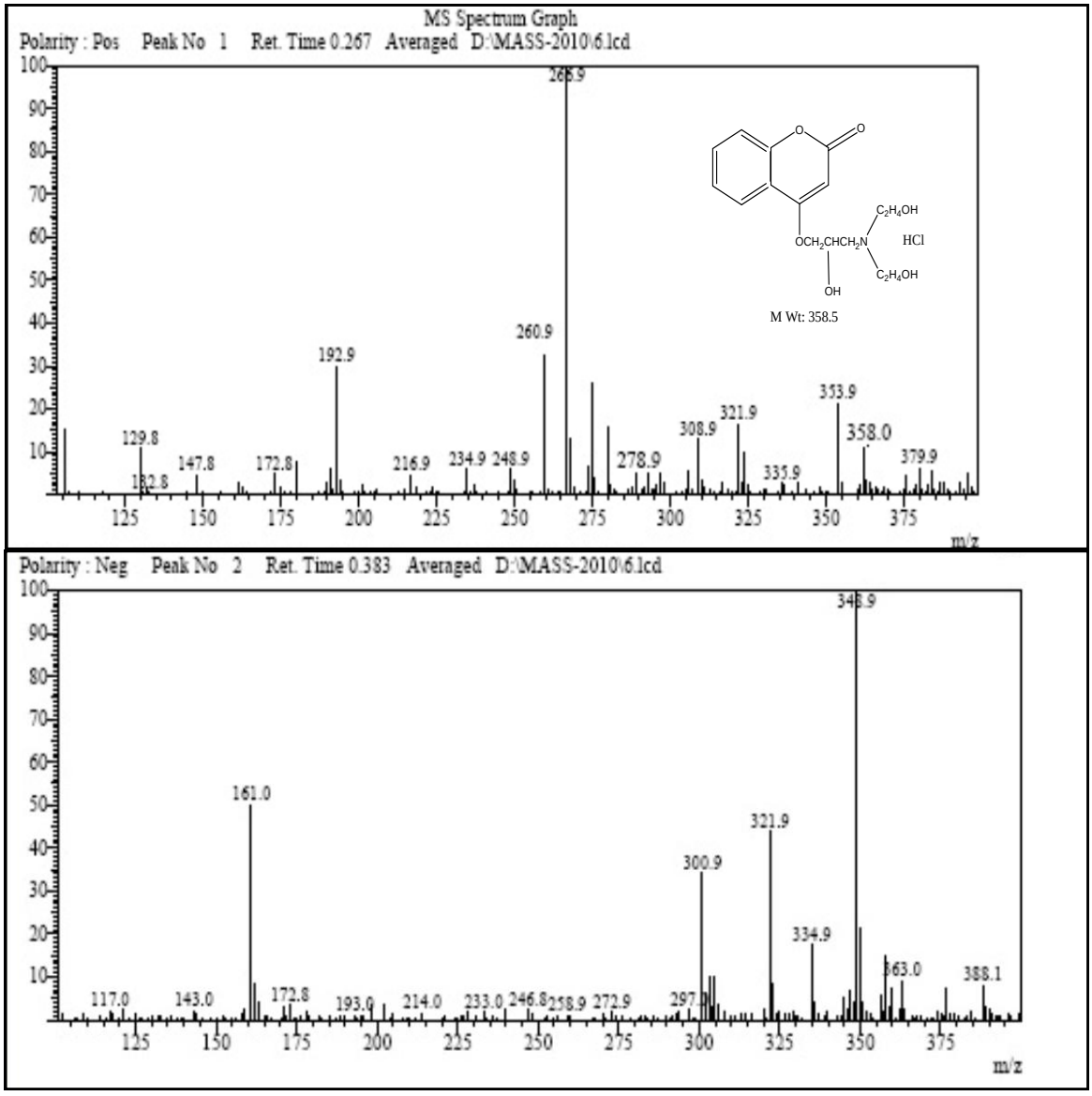
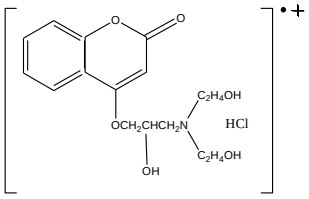
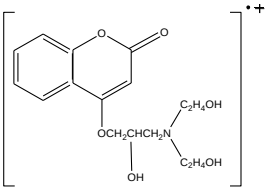
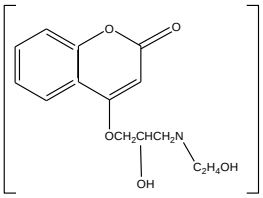
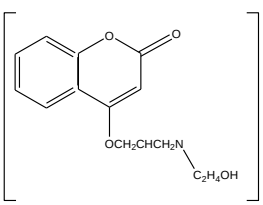
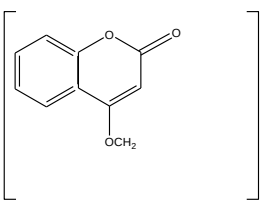
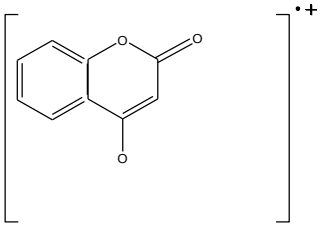



**Figure 15: Mass Spectra of BLT $_8$**

**Table 18: Mass Spectral Data of BLT $_8$**

| Sr. No. | Structure | m/e |
|---|---|---|
| 1 |  | 353.5 |
| 2 |  | 323 |
| 3 |  | 278 |
| 4 |  | 261 |
| 5 |  | 175 |
| 6 |  | 161 |

**BLT $_{18}$**

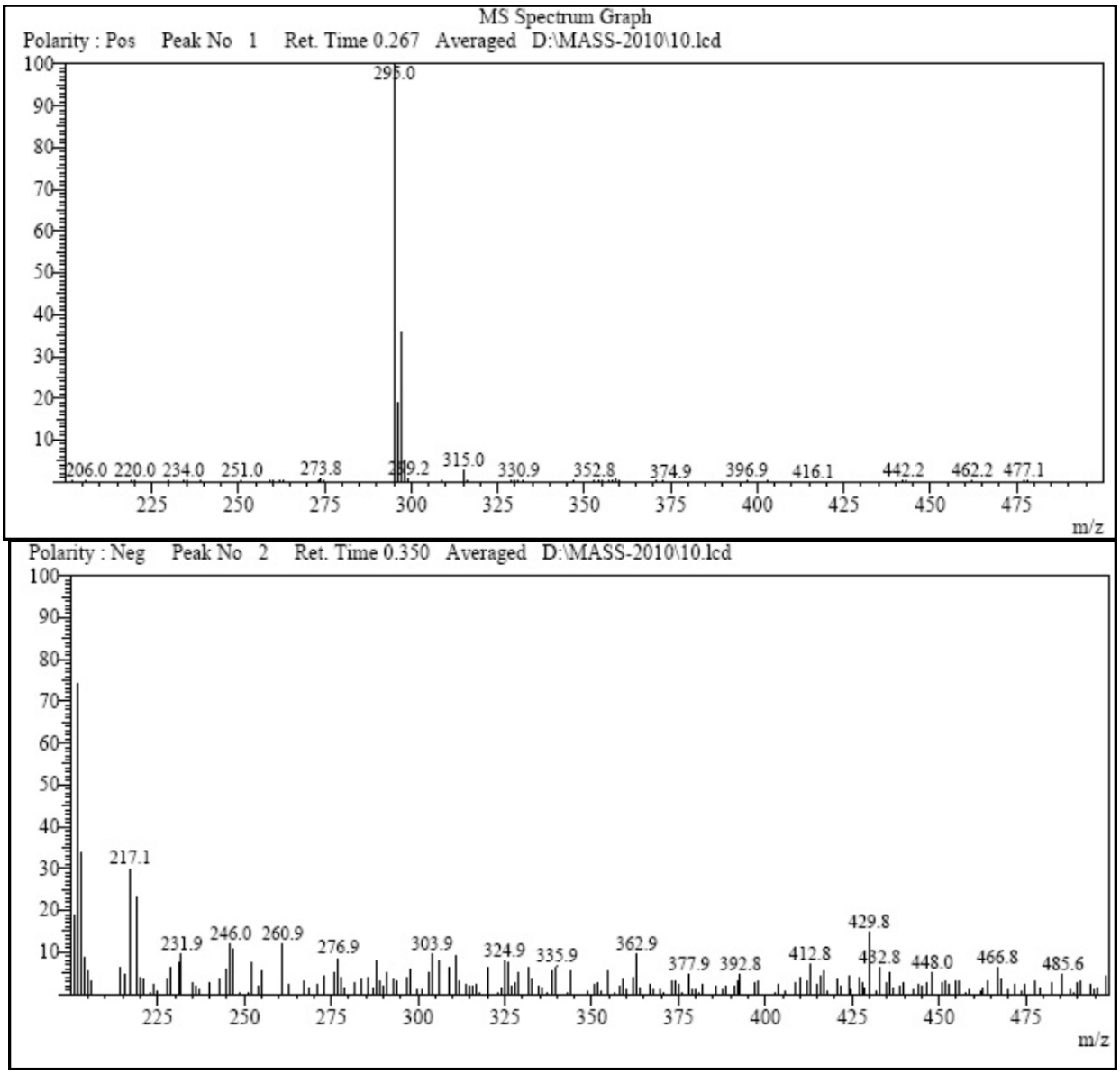
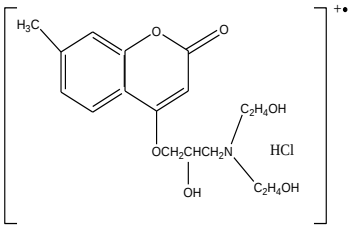
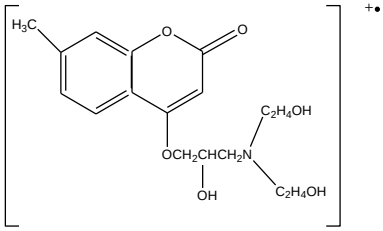
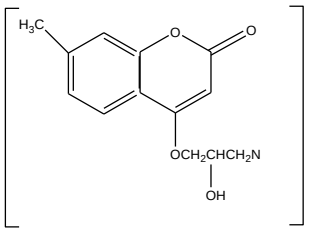
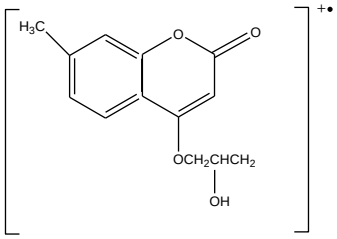
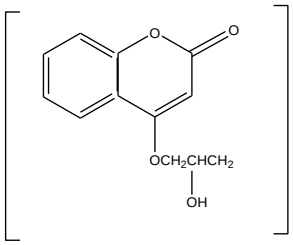



**Figure 16: Mass Spectra of BLT $_{18}$**

**Table 19: Mass Spectral Data of BLT 18**

| Sr. No. | Structure | m/e |
|---|---|---|
| 1 |  | 373.5 |
| 2 |  | 337 |
| 3 |  | 247 |
| 4 |  | 233 |
| 5 |  | 218 |

**BLT 21**

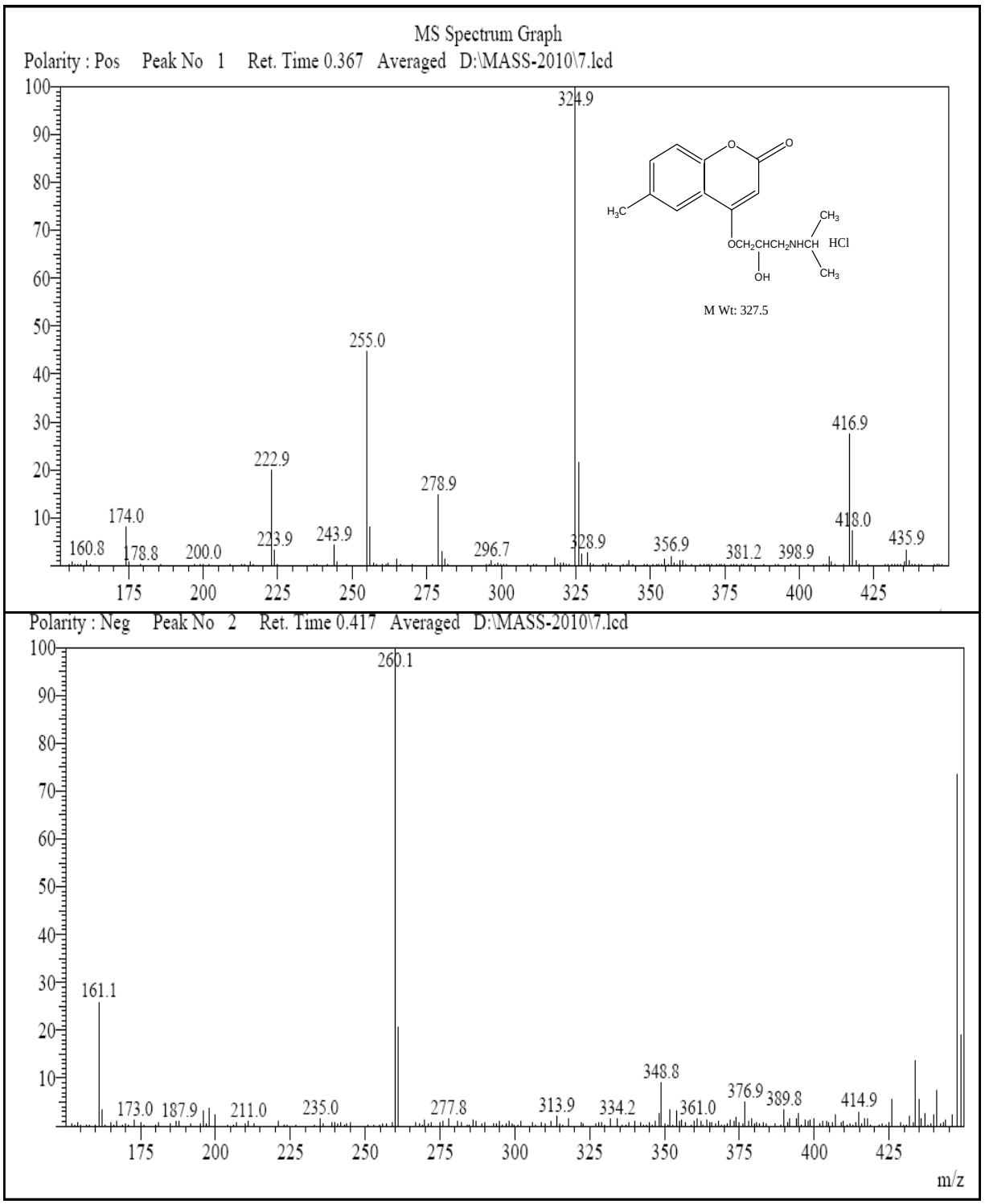
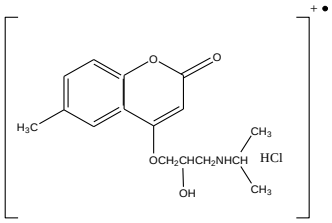
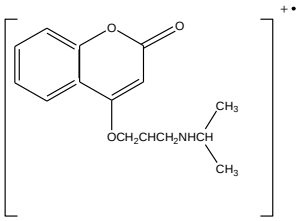
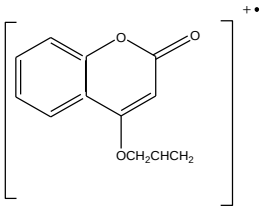
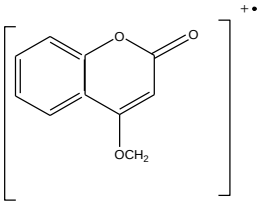
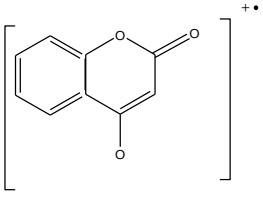



**Figure 17: Mass Spectra of BLT 21**

**Table 20: Mass Spectral Data of BLT $_{21}$**

| Sr. No. | Structure | m/e |
|---|---|---|
| 1 |  | 327.5 |
| 2 |  | 261 |
| 3 |  | 203 |
| 4 |  | 176 |
| 5 |  | 164 |

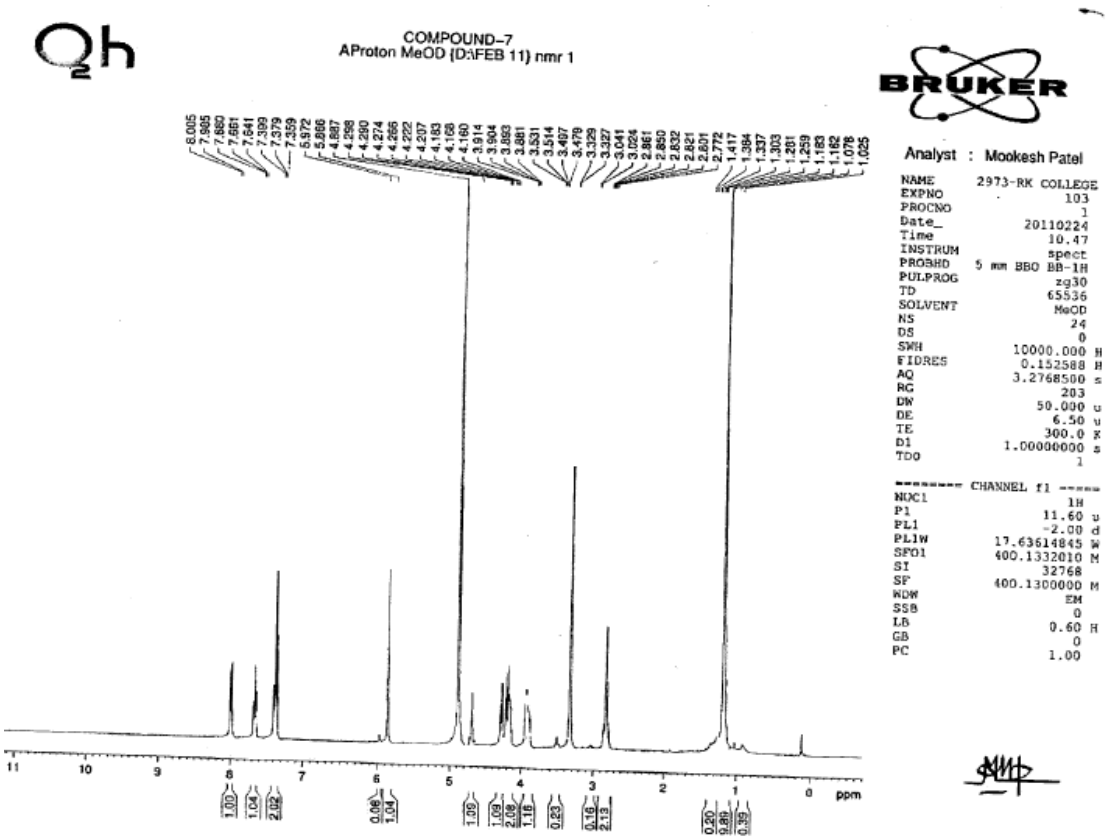



**Fig. 18 NMR Spectra of BLT $_2$**

**Table.21 NMR Spectral Data of BLT $_2$**

| Sr. No. | Signal Position (δ ppm) | Multiplicity | Inference |
|---|---|---|---|
| 1 | 1.16 | Singlet | -C<u>H</u>$_3$ |
| 2 | 2.77-2.86 | Doublet | -C<u>H</u>$_2$-NH |
| 3 | 3.88 | Singlet | -N<u>H</u> |
| 4 | 4.16-4.22 | Triplate | -C<u>H</u>$_2$CH |
| 5 | 4.26-4.29 | Multiplet | -CH$_2$C<u>H</u>CH$_2$ |
| 6 | 4.88 | Singlet | -CH in ring (coumarin) |
| 7 | 5.86 | Singlet | -OH |
| 8 | 7.35-8.00 | Multiplet | Ar-H |

## 6.3 PHARMACOLOGICAL SCREENING

### 6.3.1 Bleeding Time and Clotting Time

Warfarin- treated (0.1mg/kg p.o.) rats showed significant increase in bleeding and clotting time, as compared to normal control rats. Treatment with, all 15 test compounds (5ml/kg/day, p.o) also produced significant increase in bleeding and clotting time, as compared to normal control rats.

**Table 22: Effect of Warfarin and BLT $_{1,2,7,8,10,11,12,17,18,20,21,22,27,28,30}$ Treatment on Bleeding and Clotting Times of Rats.**

| Blood parameters | Bleeding Time (sec) | Clotting Time (sec) |
|---|---|---|
| Normal | 80+ 12 | 130+ 22 |
| Warfarin | 190+ 18 # | 390+ 35 # |
| BLT 1 | 162 ± 34 * | 331 ± 37 * |
| BLT 2 | 186 ± 27 # | 371 ± 23 # |
| BLT 7 | 143 ± 41 | 289 ± 39 |
| BLT 8 | 180 ± 35 # | 363 ± 26 # |
| BLT 10 | 133 ± 46 | 278 ± 56 |
| BLT 11 | 148 ± 25 | 310 ± 20 |
| BLT 12 | 176 ± 28 # | 365 ± 37 # |
| BLT 17 | 146 ± 37 | 322 ± 43 |
| BLT 18 | 176 ± 43 # | 362 ± 30 # |
| BLT 20 | 152 ± 24 * | 335 ± 52 * |
| BLT 21 | 145 ± 35 | 331 ± 47 * |
| BLT 22 | 170 ± 23 # | 356 ± 52 * |
| BLT 27 | 149 ± 35 * | 320 ± 37 |
| BLT 28 | 171 ± 12 # | 358 ± 29 # |
| BLT 30 | 144 ± 39 | 325 ± 43 |

Values are expressed as Mean ± S.E.M

*- significantly different from control ($p < 0.05$), # - significantly different from control ($p < 0.01$)

Above data indicates that a change in the chemical structure of coumarin does not alter its anti-coagulant activity.

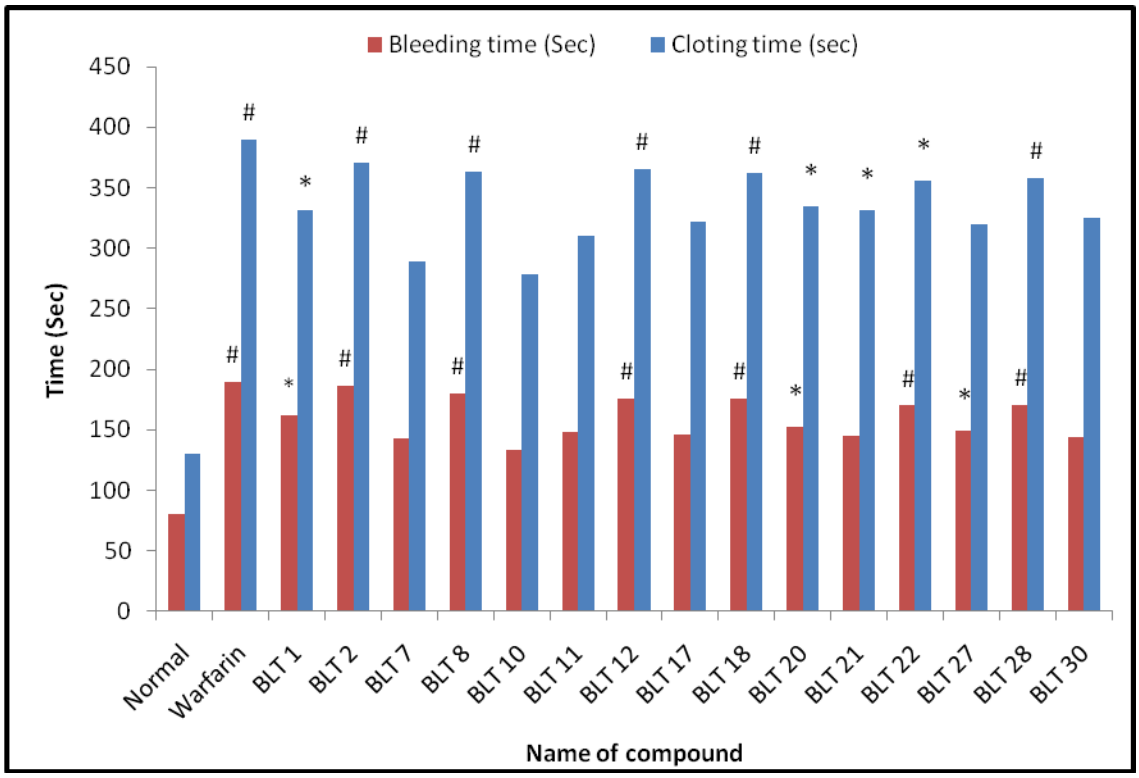



**Fig. 19 Anticoagulant Screening of Synthesized Compounds**

### 6.3.2 Antihypertensive Screening:

9 anticoagulant compounds were subjected to antihypertensive screening. Here, noradrenalin-treated (10mg/kg i. v.) rats showed significant increase in blood pressure. Treatment with test compounds (5mg/kg/day, IV) also produced antihypertensive action, but BLT 1, 2, 21 had significant effect as compared to others.

**Table 23: Pharmacological Screening of Antihypertensive Activity**

| Drug | Blood pressure (mmHg) | | | |
|---|---|---|---|---|
| | Normal | Nor-adrenaline (NA) | Test compound + NA | Difference |
| **BLT-1** | 120 | 208 ± 5 | 166 ± 14 * | 42 |
| **BLT-2** | 122 | 215 ± 7 | 173 ± 13 * | 42 |
| **BLT-7** | 116 | 210 ± 6 | 187 ± 17 | 23 |
| **BLT-11** | 122 | 210 ± 10 | 192 ± 15 | 18 |
| **BLT-12** | 120 | 208 ± 7 | 180 ± 14 | 28 |
| **BLT-17** | 120 | 236 ± 5 | 208 ± 16 | 28 |
| **BLT-21** | 120 | 208 ± 8 | 154 ± 17 * | 54 |
| **BLT-22** | 120 | 208 ± 9 | 180 ± 18 | 28 |
| **BLT-27** | 120 | 236 ± 11 | 210 ± 16 | 26 |

***- significantly different from control ($p < 0.05$)

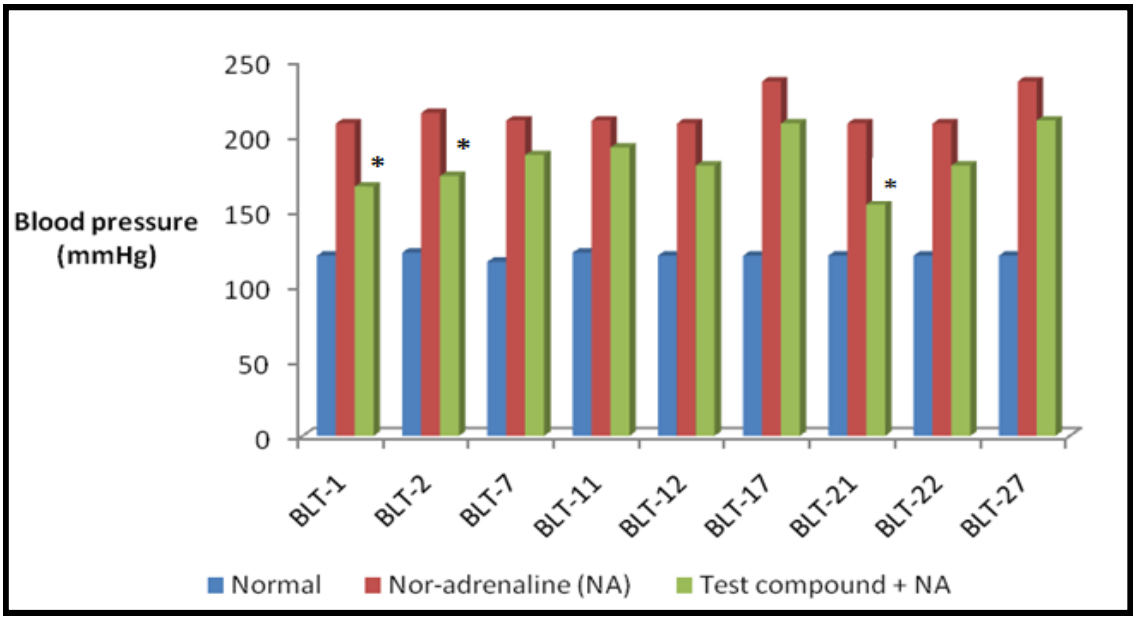
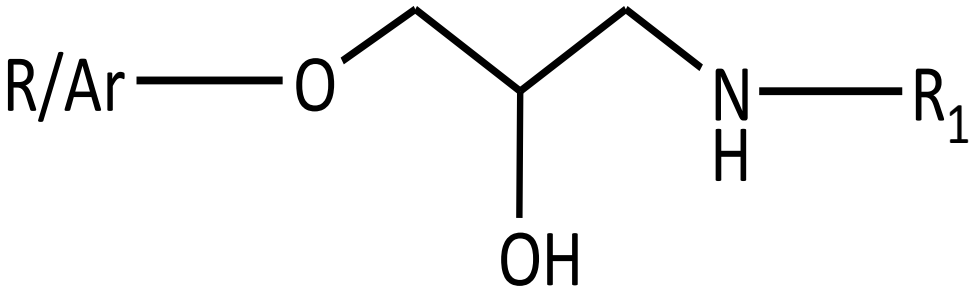



**Figure 21: Pharmacological Screening of Antihypertensive Activity**

We infer that, Isopropylamine side chain may play an important role in antihypertensive activity of the compound.




# DISCUSSION

# 6. DISCUSSION

Since, hypertension and thrombo-embolism are intricately involved in pathophysiological changes and progression of cardio-vascular diseases; antihypertensive drugs like propranolol and anticoagulant drugs like warfarin (a coumarin derivative) are frequently, combined in management of some types of hypertension. Therefore, in the present project, we attempted to synthesize a single drug molecule with antihypertensive activity of propranolol and anticoagulant activity of Warfarin. To achieve this, we attached alkylaminohydroxypropoxy side chain (responsible for antihypertensive activity of propranolol) to 4-Hydroxy coumarin nucleus (responsible for anticoagulant activity of warfarin).

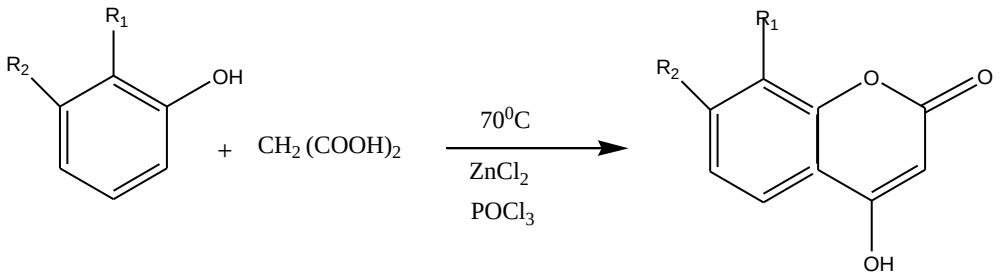
In our project, for synthesis of substituted 4-hydroxy coumarin nucleus, we used phenol, m-cresol and p-cresol as starting materials. Thus, we synthesized 3 basic coumarin nucleus viz. 4-hyddroxy coumarin, 7-methyl-4-hydroxy coumarin and 8-methyl-4-hydroxy coumarin nucleus, respectively. Each nucleus was then, reacted with epichlorhydrin to form three varieties of epoxy derivatives. Finally, each epoxy derivative was reacted with 10 different amines to form 10 different coumarin molecules. All these, 30 compounds were then, converted to hydrochloride salts by reacting them with dry hydrochloric acid gas.

The syntheses involved following steps:

1. Preparation of 4-hydroxy coumarin nucleus
2. Preparation of 4-(2, 3-epoxypropoxy) coumarin
3. Preparation of 4-(2-hydroxy-3-alkylaminopropoxy) coumarin
4. Preparation of 4-(2-hydroxy-3-alkylaminopropoxy) coumarin hydrochloride

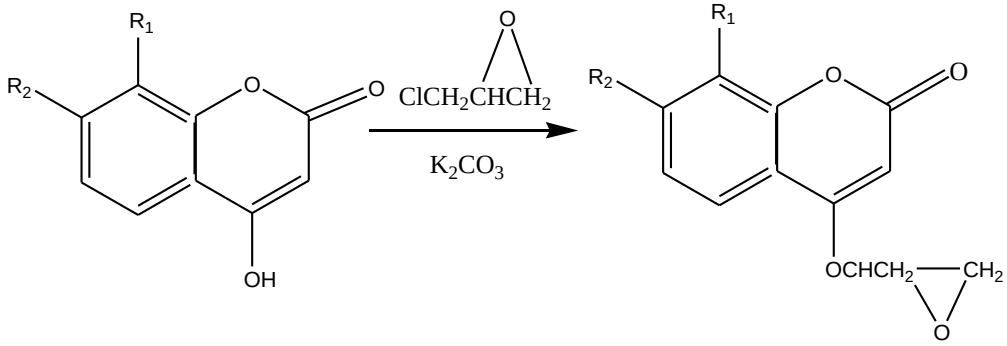
**1. Preparation 4-hydroxy coumarin nucleus**

AS per literature report, we found that phenol reacts with malonic acid and phosphorusoxychloride in presence of anhydrous zinc chloride give 4-hydoxy coumarin.



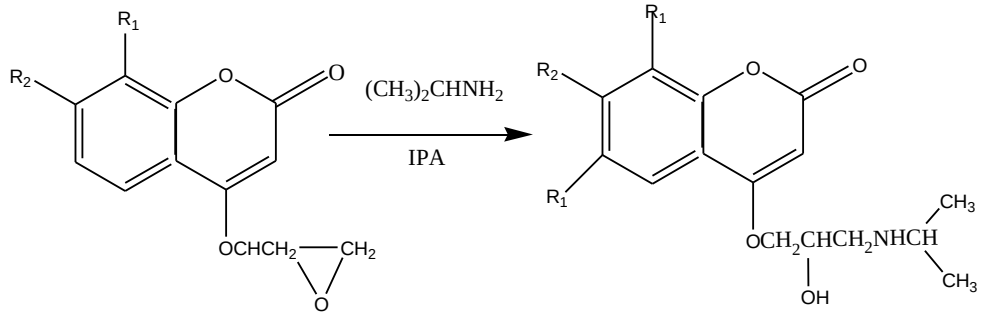

For synthesis of 4-hydroxy coumarin, we followed new method developed by Indian scientist Bose *et al.* They synthesized 4-hydroxy coumarins by heating diaryl malonates with equimolor moles of corresponding malonic acid in the presence of about anhydrous $ZnCl_2$ and $POCl_3$. We attempted to carry out this reaction at $70^0$C for 12-15 hours, but it was observed that maximum yield was obtained only after 16 hours. It was also noted that on acidification of sodium bicarbonate filtrate, at neutral point, the product precipitated out. The yield was increased, if acidified slurry was kept overnight. Hence, this modified version of reaction was adopted for synthesis of all other coumarin derivatives in our project. Our attempts to carry out reactions of other phenols like α-napthol, β-napthol and hydroxyl benzoic acid were unsuccessful.

**2. Preparation of 4-(2, 3-epoxypropoxy) coumarin**



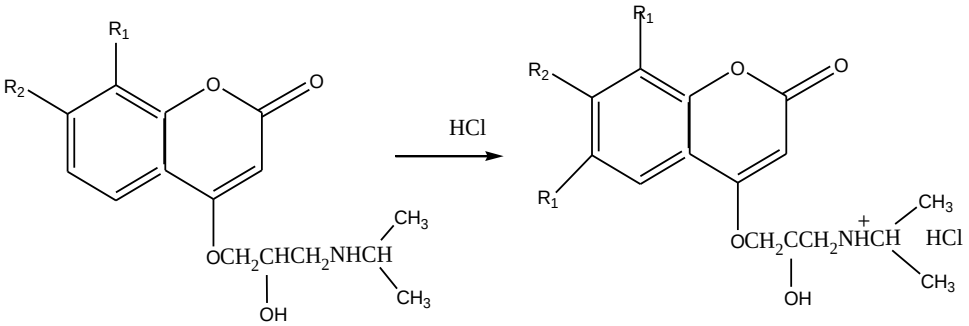

When 4-hydroxy coumarin derivative was treated with epichlorhydrine in presence of base, under suitable condition, resulted in formation of the epoxy derivative. To obtain solid product, the epoxy resin was completely separated from reaction mixture, by using mother solvent i.e. toluene. This was dissolved in a minimum quantity of dioxane and was slowly poured on to crushed ice with vigorous stirring. Thus, solid epoxy derivative was isolated. Attempt to use benzene instead of toluene failed to produce a solid product.

### 3. Preparation of 4-(2-hydroxy-3-isopropylaminopropoxy) coumarin




In this step, the epoxy derivative was treated with an amine to form a product. This reaction was also successful if Iso- Propyl Alcohol (IPA) was used as a solvent. Semisolid mass was obtained only, when the solvent or unreacted amine was distilled out. When this product was dissolved in 1:5 mixture of DMF + dioxane then poured on to ice + water mixture, a free product (base) in aqueous medium obtained. But when it was filtered, it soon converted in to brown semisolid mass.

### 4. Preparation of 4-(2-hydroxy-3-isopropylaminopropoxy) coumarin hydrochloride




The base was dissolved in appropriate solvent and converted in to hydrochloride salt and isolated as a white solid product. To dissolve semisolid base, we used different solvent mixtures, in different proportions and then treated with dry hydrochloric acid gas to obtain the corresponding free hydrochloride. Solvent mixtures used were (A) Methanol + Diethylether (B) Isopropanol + Ethylacetate (C) Isopropanol + Diethylether (D) Isopropanol + Diethylether. But all these solvent mixtures proved to be unsuccessful except, Isopropanol + Diethylether (1:3) mixture.

Synthesized compounds conformed to the spectral analysis. IR spectral characteristic peak of carbonyl group, in coumarin moiety, was observed at 1700-1722 $cm^{-1}$frequency, while C-

O streching of ring skeleton was observed at 1160-1125 $cm^{-1}$ frequency. The N-H streching of secondary amines gave a broad peak between 3350-3300 $cm^{-1}$ frequency. The molecular ion peak and the base peak, in all compounds, were clearly obtained in mass spectral study. NMR Spectra of compound $BLT_{2,}$ were taken which showed methylene ($-CH_2$), amine (>NH), hydroxy (R-OH) methyl ($-CH_3$) and aromatic protons (Ar-H) etc. peaks.

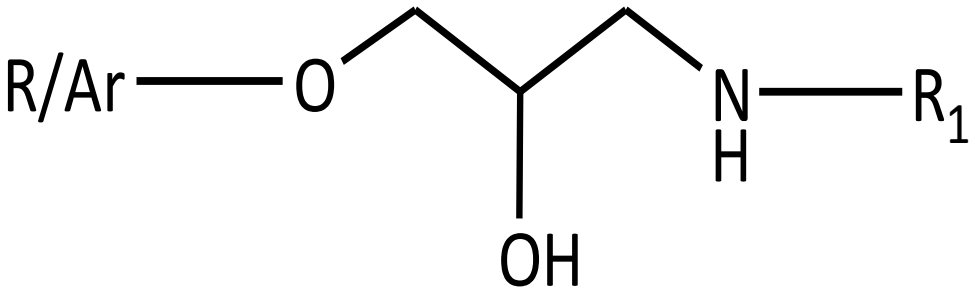
When subjected to pharmacological screening for anticoagulant activity and antihypertensive activity; all 12 synthesized compounds exhibited anticoagulant activity but highly significant anticoagulant activity was observed in compounds BLT $_{2,\ 8,\ 12,\ 18,\ 28}$ accompanied by increased bleeding and clotting time that was near to the standard drug viz. Warfarin, indicating that 6-methyl and 7-methyl derivatives also possess anticoagulant activity. Substituted groups that contain four carbon atoms, if substituted at nitrogen atom, exhibit maximum activity. This goes to confirm that 4-hydroxycoumarin derivatives, have anticoagulant property and that there is no relation between substituted and unsubstituted coumarin derivatives. We believe that in coumarin class anticoagulant compounds, hydroxyl group of 4-Hydroxy coumarin plays important role in formation of cyclic structure through hydrogen bond, with side chain attached to $3^{rd}$ carbon in coumarin nucleus eg. warfarin. In case of our compounds, hydrogen bond formation takes place between hydroxyl group of side chain and pi-electrons of alkene in coumarin ring ($3^{rd}$ and $4^{th}$ carbons) hence; cyclic structure is formed showing activity in all compounds that were tested.

We believe that bulkier substitution on $3^{rd}$ position of coumarin causes increase in anticoagulant activity, e.g. ferulenol. In our project, we found that more bulky groups like tert-butyl (BLT 1) showed significant anticoagulant activity. Diethanol derived compounds BLT 8 and BLT 18, showed good activity for the same reason. Finally, we conclude that formation of ring structure in coumarin through hydrogen bonding and bulky groups at terminal part of the side chain are responsible for anticoagulant activity, in a compound.

Compounds BLT $_{1,\ 2,\ 7,\ 11,\ 12,\ 17,\ 21,\ 22,\ 27}$ were evaluated for antihypertensive activity, by using invasive method on BioPac instrument. Results showed that Isopropyl and *tert*-butyl substituted compounds i.e. BLT $_{1,\ 2,\ 21}$ possess significant antihypertensive activity. We infer

that increase in the carbon length more than four on amine substitution causes decrease in antihypertensive activity.

Thus, we synthesized 30 coumarin derivative compounds, out of which, 12 compounds exhibited anticoagulant activity, 9 among which also exhibited antihypertensive activity. Some of these 9 compounds, with dual activity, were 7-methyl substituted coumarin and 8-methyl coumarin too. 3 compounds among these were Isopropyl and tert-butyl substituted compounds possessing significant antihypertensive activity.

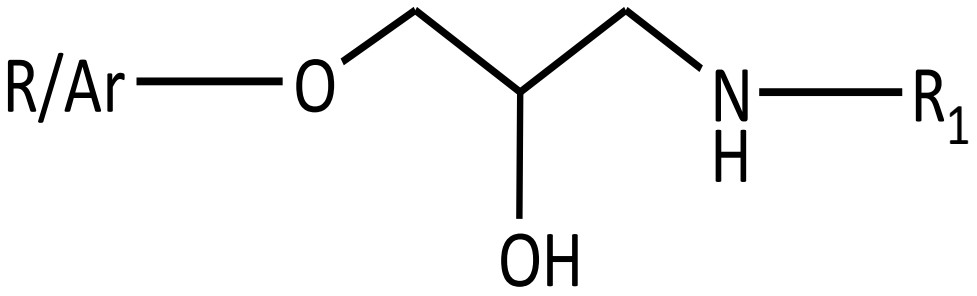



# CONCLUSION

# 7. CONCLUSION

In the present project, we have synthesized 30 coumarin derivative compounds, out of which, 12 compounds exhibited anticoagulant activity, 9 among which also exhibited antihypertensive activity. Some of these 9 compounds, with dual activity, were 7-methyl substituted coumarin and 8-methyl coumarin too. 3 compounds among these were Isopropyl and tert-butyl substituted compounds possessing significant antihypertensive activity. Literature review reveals that carbon no. 7 in coumarin nucleus of Warfarin is involved in its metabolism, before being excreted in urine. Hence, metabolism studies of these compounds may be planned as an extension of our work.

In the light of above work, we conclude that in coumarin class anticoagulant compounds, hydroxyl group of 4-Hydroxy coumarin plays important role in formation of cyclic structure through hydrogen bond, with side chain attached to $3^{rd}$ carbon in coumarin nucleus e.g. Warfarin. We also infer that formation of ring structure in coumarin through hydrogen bonding and bulky groups at terminal part of the side chain are responsible for anticoagulant activity, in a compound. Our results also indicate that increase in the carbon length more than four on amine substitution causes decrease in antihypertensive activity.

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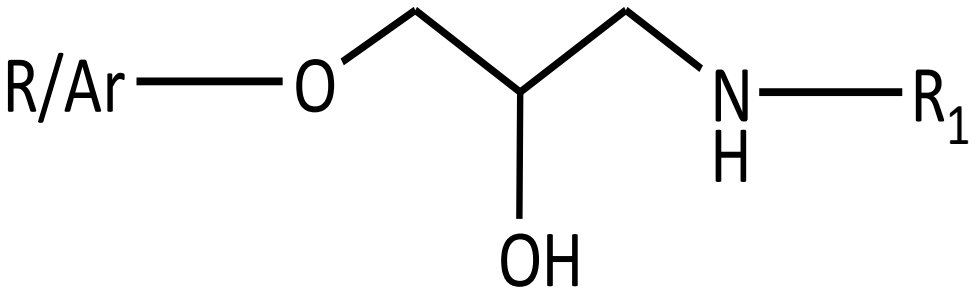


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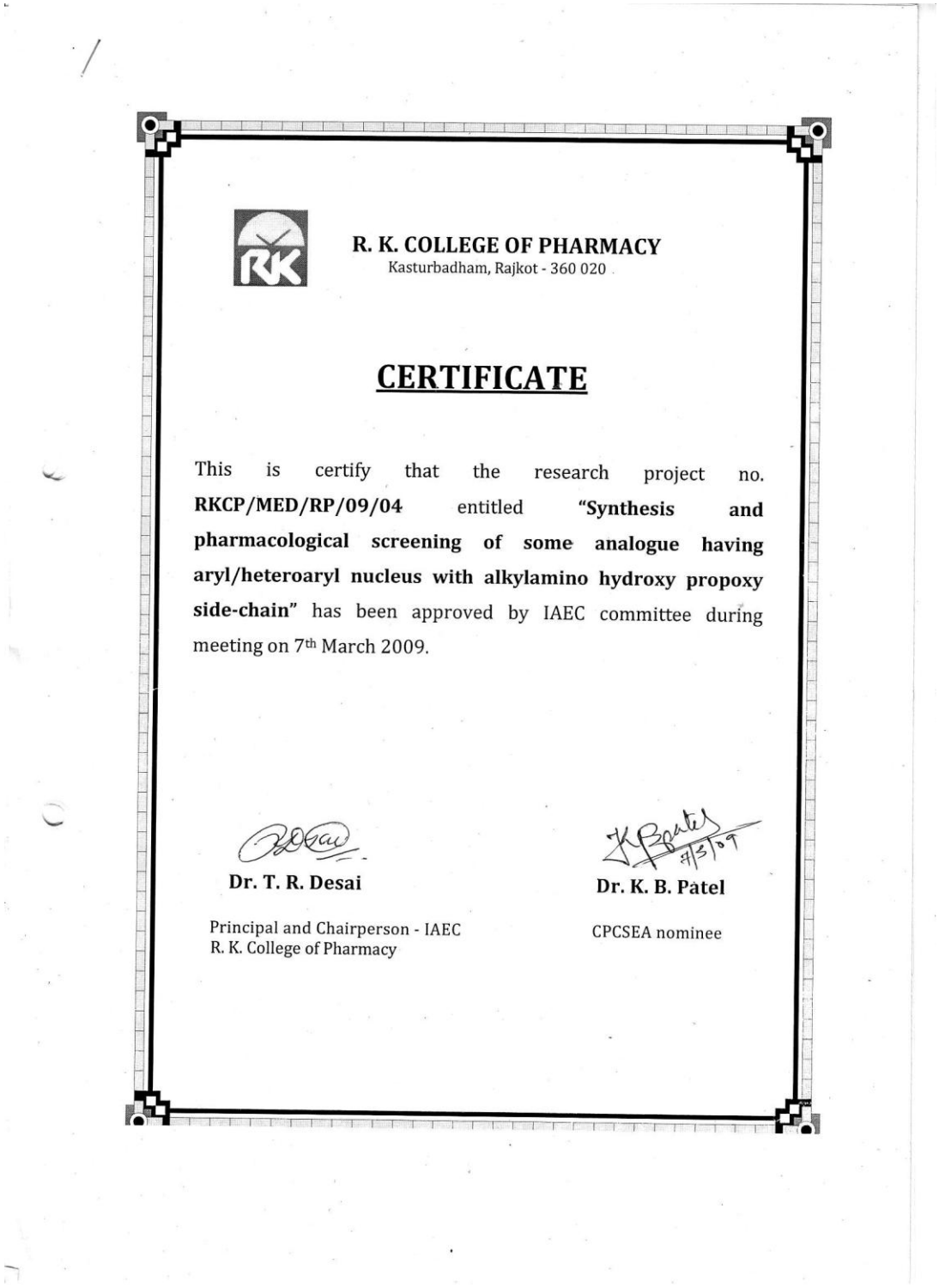

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**Dr. K. B. Patel**
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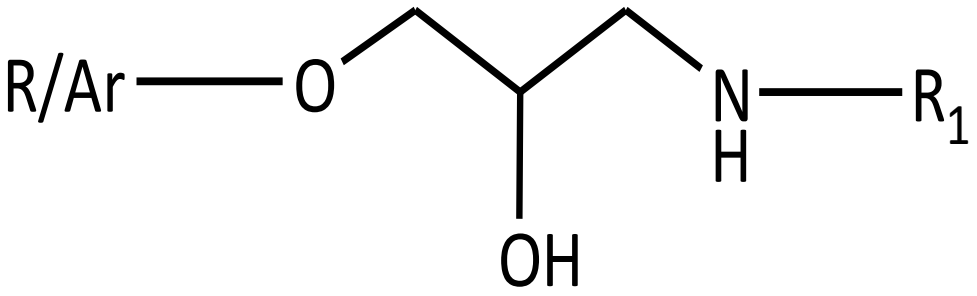


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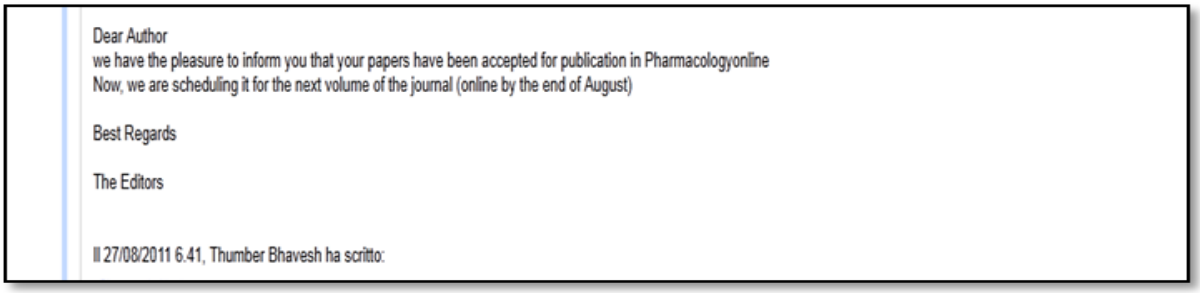

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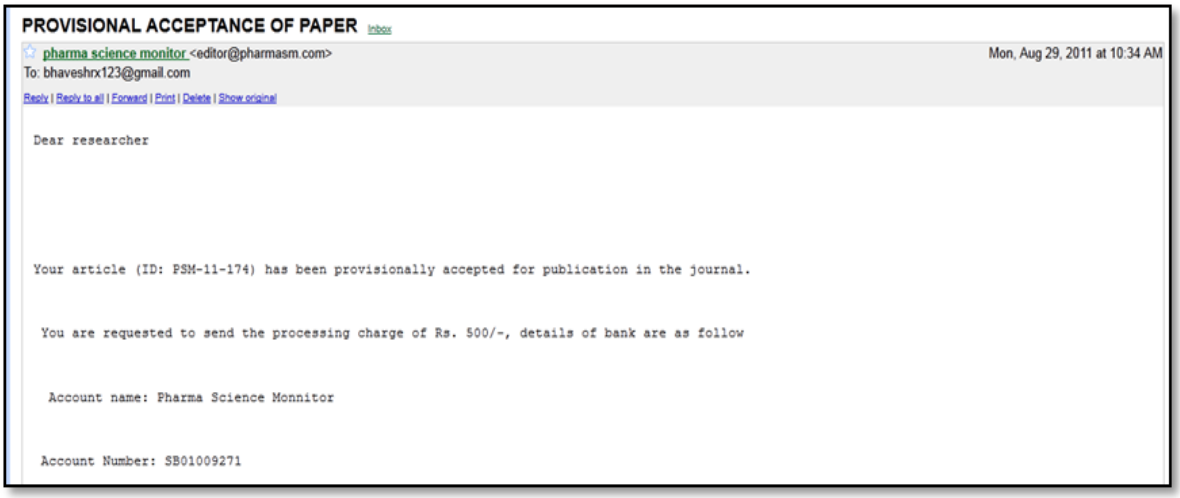

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# ANTICOAGULANT ACTIVITY OF SUBSTITUTED HYDROXY PROPOXYCOUMARIN DERIVATIVES

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## Summary

Coumarin molecule is used as anticoagulant. Other uses like anti-HIV, anti-tumor, anti-hypertensive, anti-arrhythmia, anti-osteoporosis, pain relief increase research of coumarin derivatives. Perkin discovered the synthetic method of coumarin and opened the door in synthetic research. Warfarin is a well known drug of 4-hydroxy coumarin derivative. It is consider that, warfarin drug act as a lead molecule of anticoagulant. To introduce alkylaminohydroxypropoxy side chain in coumarin nucleus changes its anticoagulant activity. Hence we have synthesized substituted coumarin derivatives and performed its anticoagulant activity.

## Key words

Warfarin, Coumarin derivative, Anticoagulant

## Introduction

Thromboembolism is the combination of thrombosis and its main complication, embolism. When a thrombus occupies more than 75% of surface area of the lumen of an artery, blood flow to the tissue supplied is reduced enough to cause symptoms because of decreased oxygen (hypoxia) and accumulation of metabolic products like lactic acid. More than 90% obstruction can result in anoxia, the complete deprivation of oxygen, and infarction, a mode of cell death [1].

The symptoms of a thromboembolism depend on the organ or blood vessel that has lost blood supply. Blood clots in an arm or leg may cause pain, swelling, and increased temperature in the affected area. A clot that travels to the lung is called a pulmonary embolus. This condition can cause: chest pain, shortness of breath, rapid heartbeat, known as tachycardia, fainting or death. If a blood clot is formed in the heart, it can travel to almost any organ in the body. This could cause a stroke, which is a type of damage to the brain from lack of blood circulation. In other cases, damage may be done to an arm or leg, or a heart attack or kidney damage may occur. Other areas of the body can also be affected [2].

An anticoagulant is a substance that prevents coagulation; that is, it stops blood from clotting. Anticoagulants were introduced into medical practice more than three decades ago. Extensive use of these drugs in the prevention and treatment of thromboembolic disease has made them one of the most widely used classes of pharmacological agents.

Anticoagulant drugs include: Heparin and derivative substances e.g. Low molecular weight heparin and Coumarins (Vitamin K antagonists) e.g. Warfarin [3].

Warfarin acts via inhibition an enzyme vitamin K epoxide reductase, which recycles oxidized vitamin K to its reduced form after it has participated in the carboxylation of several blood coagulation proteins, mainly prothrombin and factor VII [4]. Reduced vitamin K must be regenerated from the epoxide for sustained carboxylation and synthesis of biologically competent proteins [5]. So we have tried to synthesize coumarin derivative which posse's anticoagulant activity.

Coumarin derivatives are used as therapeutic anticoagulants and as rodenticides by causing fatal haemorrhage [6]. Because the range between efficient therapy and undue hemorrhagic risk may vary greatly from one patient to another, the need for carefully individualized treatment and frequent observations has long been stressed. However, a summary of recent research findings, along with certain principles, may offer possible explanations for responsiveness to make highly efficient lead with fewer side effects to resist both, coagulopathy as well as hypertension. The primary aim of this present work is to study pharmacological screening and synthetic aspects of the coumarin ring structure especially its combined analogues profile as an anticoagulant and antihypertensive property. Our first aim is to study anticoagulant activity of synthesized compounds and then work on antihypertensive parameters.

## Materials and Method

Sterile disposable pricking needle or lancet, stop watch, dry glass capillary tube (narrow diameter 1 top 2 mm, minimum 10 cm long), cotton swab of absorbent cotton, spirit wetted. 70 % v/v ethyl alcohol or 70 % v/v denatured spirit is used as antiseptic.

Blood sample collection and blood analysis: Blood samples were collected in clean dry centrifuge tubes as end of three weeks of treatment after 12hrs fast from retro orbital plexuses under light ether anesthesia and were collected in EDTA tube to prevent clot formation at room temperature.

Determination of clotting time using Lee and white method.

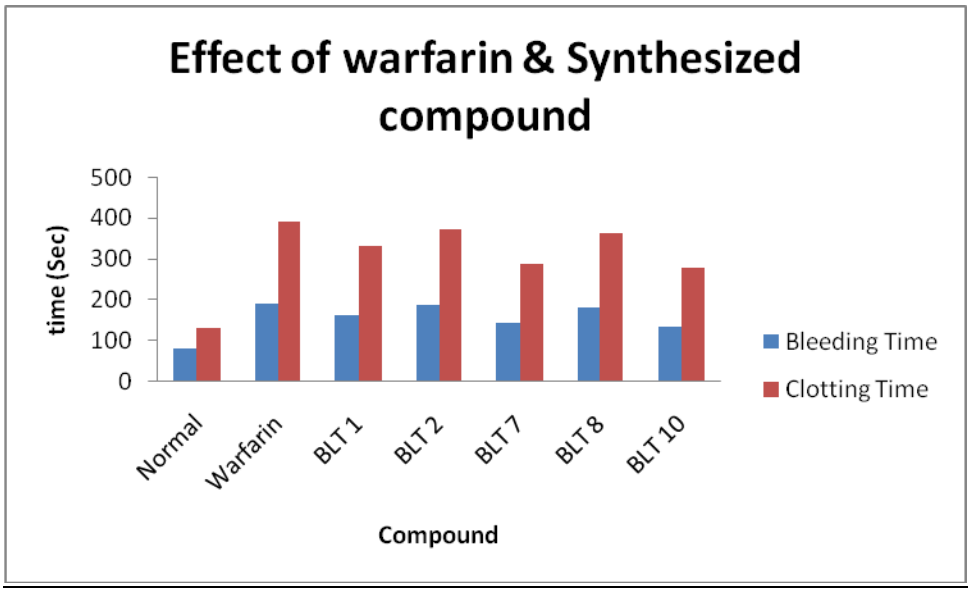
## Experimental:

We have synthesized a series of 4-substituted coumarin derivatives and performed its pharmacological screening for anticoagulant activity. $4^{th}$ position is substituted with alkylaminohydroxypropoxy side chain.

Blood was collected from animal by retro orbital plexus method under light anesthetic conditions. Immediately stop watch was started. Dip one end of capillary into blood drop gently without pressure. After every 30 seconds, using stopwatch, break a small piece of capillary. Repeat breaking at regular time intervals, till fibrin thread appears at broken end of capillary tube. Do not pull away the cut pieces ling apart and bristly. Record time interval between pricking finger and first appearance of fibrin thread at the broken ends of capillary tube. That is clotting time of blood.

Statistical analysis: Results are presented as mean ± SEM. Statistical differences between the means of the various groups were evaluated using one-way analysis of variance (ANOVA) followed by Tukey's test. Data were considered statistically significant at $P \leq 0.05$ and highly significant at $P \leq 0.001$. Statistical analysis was performed using Sigma stat statistical software.

## Result and Discussion




Warfarin treated (0.1mg/kg p.o.) rats were found to be shown significant increase in bleeding and clotting time as compare to normal healthy rat. Treatment with tested compounds (5ml/kg/day, p.o) also produced significant increase in bleeding and clotting time as compared to normal rats. It may be act same as warfarin, but it is confirmed that change in chemical structure of coumarin side chain altered its anti-coagulant activity. Coumarin nucleus is responsible for anticoagulant activity. While side chain play important role in other activity like hypertension, arrhythmia etc. Our first aim is synthesized anticoagulant compound which posses antihypertensive activity. Primary screening for anticoagulant activity is performed while other activity will perform as early as possible.

## Acknowledgement

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# ANTICOAGULANT ACTIVITY OF METHYLATED COUMARIN DERIVATIVES

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## Summary

Tremendous research on coumarin derivatives and anticoagulant drug are main inspiration of this work. Methylated coumarin derivative substituted by alkylaminohaydroxypropoxy side chain gives antihypertensive as well as anticoagulant activities. Therefore, in the present project, we attempted to synthesize a single drug molecule with antihypertensive activity of propranolol and anticoagulant activity of warfarin (coumarin).

## Key words

Anticoagulant, methylated coumarin, antihypertensive

## Introduction

It may be a first attempt to work on coumarin derivative as antihypertensive agent. Blood is a specialized bodily fluid that delivers necessary substances to the body's cells – such as nutrients and oxygen – and transports waste products away from those same cells. Blood is composed of blood cells suspended in a liquid called blood plasma. Plasma, which comprises 55% of blood fluid, is mostly water (90% by volume) and contains dissolved proteins, glucose, mineral ions, hormones, carbon dioxide (plasma being main medium for excretory product transportation), platelets and blood cells themselves. The blood cells present in blood are mainly red blood cells (also called RBCs or erythrocytes) and white blood cells, including leukocytes and platelets. The most abundant cells in human blood are red blood cells. These contain hemoglobin, an iron-containing protein, which facilitates transportation of oxygen by reversibly binding to this respiratory gas and greatly increasing its solubility in blood. In contrast, carbon dioxide is almost entirely transported extracellular dissolved in plasma as bicarbonate ion [1].

Blood diseases affect the production of blood and its components, such as blood cells, hemoglobin, blood proteins, mechanism of coagulation, etc. Thrombosis is formation of a blood clot (thrombus) inside a blood vessel, obstructing flow of blood through circulatory system. When a blood vessel is injured, body uses platelets and fibrin to form a blood clot, because first step in repairing it (hemostasis) is to prevent loss of blood. If that mechanism causes too much clotting, and clot breaks free, an embolus is formed**.** This plug obstructs normal flow of blood and can result in a heart attack or stroke [2].

Warfarin is coumarin containing anticoagulant. The story of the coumarin anticoagulants generally is traced back to the early 1920s, when the "sweet clover disease" showed up almost simultaneously in North Dakota and in Alberta, Canada. This new malady of cattle involving fatal bleeding was traced to stacks of sweet clover hay [3]. An anticoagulant is a substance that prevents coagulation; that is, it stops blood from clotting. Anticoagulants were introduced into medical practice more than three decades ago. Extensive use of these drugs in the prevention and treatment of thromboembolic disease has made them one of the most widely used classes of pharmacological agents. Antiplatelet agents, anticoagulant agents and thrombolytic drugs use as antithrombic agent.

Coumarins are a group of important natural compounds, and have been found to have multi-biological activities such as anti-HIV, anti-tumor, anti-hypertensive, anti-arrhythmia, anti-osteoporosis, pain relief, preventing asthma and antisepsis [4]. Natural products like esculetin, fraxetin, daphnetin and other related coumarin derivatives are recognized as inhibitors not only of the lipoxygenase and cycloxygenase enzymic systems, but also of the neutrophil-dependent superoxide anion generation. Coumarin derivatives also possess anti-inflammatory as well as antioxidant activities. Coumarin possesses immunomodulatory and direct antitumor activity [5]. It has been recommended for treatment of a number of clinical conditions, including high protein oedema and brucellosis. Coumarin and some of its derivatives have been tested for treatment of anxiolytic, microcirculation disorders and angiopathic ulcers, and also for treatment of high protein oedemas in animals [6].

## Materials and Methods

Sterile disposable pricking needle, stop watch, dry glass capillary tube (narrow diameter 1 top 2 mm, minimum 10 cm long), cotton swab, spirit wetted, 70 % v/v ethyl alcohol.

Blood sample collection and blood analysis: Blood samples were collected in clean dry centrifuge tubes as end of three weeks of treatment after 12hrs fast from retro orbital plexuses under light ether anesthesia and were collected in EDTA tube to prevent clot formation at room temperature.

We have used Lee and white method for determination of clotting time.

## Experimental

We have synthesized a series of methylated coumarin substituted derivatives and performed its pharmacological screening for anticoagulant activity. $4^{th}$ position is substituted with alkylaminohydroxypropoxy side chain as in propranolol.

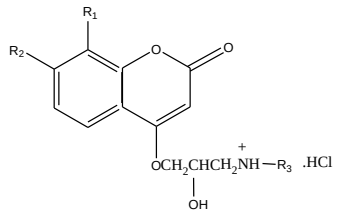
Blood was collected from animal by retro orbital plexus method under light anesthetic conditions. Immediately stop watch was started. Dip one end of capillary into blood drop gently without pressure. After every 30 seconds, using stopwatch, break a small piece of capillary. Repeat breaking at regular time intervals, till fibrin thread appears at broken end of capillary tube. Do not pull away the cut pieces ling apart and bristly. Record time

interval between pricking finger and first appearance of fibrin thread at the broken ends of capillary tube. That is clotting time of blood.

Statistical analysis: Results are presented as mean ± SEM. Statistical differences between the means of the various groups were evaluated using one-way analysis of variance (ANOVA) followed by Tukey's test. Data were considered statistically significant at $P \leq 0.05$ and highly significant at $P \leq 0.001$. Statistical analysis was performed using Sigma stat statistical software.

## Result and Discussion

We have synthesized a series of coumarin derivative. This may possess antihypertensive activity too.




**Tab: 1** Synthesized compounds

| Sr. No | Code | $R_1$ | $R_2$ | $R_3$ | M.F. | M. P. (°C) | $R_{f*}$ value | % Yield |
|---|---|---|---|---|---|---|---|---|
| 1 | BLT 11 | $-CH_3$ | H | $-CH(CH_3)_2$ | $C_{16}H_{22}NO_4$ Cl | 245-47 | 0.31 | 56 |
| 2 | BLT 12 | $-CH_3$ | H | $-C(CH_3)_3$ | $C_{17}H_{24}NO_4$ Cl | 240-42 | 0.30 | 59 |
| 3 | BLT 17 | $-CH_3$ | H | $-(C_2H_5)_2$ | $C_{17}H_{24}NO_4$ Cl | 270-72 | 0.31 | 50 |
| 4 | BLT 18 | $-CH_3$ | H | $-(C_2H_40H)_2$ | $C_{17}H_{24}NO_6$ Cl | 258-60 | 0.40 | 38 |
| 5 | BLT 20 | $-CH_3$ | H | $-(CH_3)_2$ | $C_{15}H_{20}NO_4$ Cl | 262-64 | 0.31 | 38 |
| 6 | BLT 21 | H | $-CH_3$ | $-CH(CH_3)_2$ | $C_{16}H_{22}NO_4$ Cl | 251-53 | 0.31 | 50 |
| 7 | BLT 22 | H | $-CH_3$ | $-C(CH_3)_3$ | $C_{17}H_{24}NO_4$ Cl | 244-46 | 0.24 | 48 |
| 8 | BLT 27 | H | $-CH_3$ | $-(C_2H_5)_2$ | $C_{17}H_{24}NO_4$ Cl | 278-80 | 0.35 | 48 |
| 9 | BLT 28 | H | $-CH_3$ | $-(C_2H_40H)_2$ | $C_{17}H_{24}NO_6$ Cl | 258-60 | 0.41 | 38 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 10 | BLT 30 | H | $-CH_3$ | $-(CH_3)_2$ | $C_{15}H_{20}NO_4$ Cl | 265-67 | 0.25 | 36 |

Warfarin treated (0.1mg/kg p.o.) rats were found to be shown significant increase in bleeding and clotting time as compare to normal healthy rat. Treatment with tested compounds (5ml/kg/day, p.o) also produced significant increase in bleeding and clotting time as compared to normal rats. We have not found it's mechanism but it is confirmed that change in chemical structure of coumarin side chain altered its anti-coagulant activity. Coumarin nucleus is responsible for anticoagulant activity. While side chain play important role in other activities like hypertension, arrhythmia etc. Our first aim is synthesized anticoagulant compound which posses antihypertensive activity. Our work may helpful to discover the new series of drug use in hypertension.

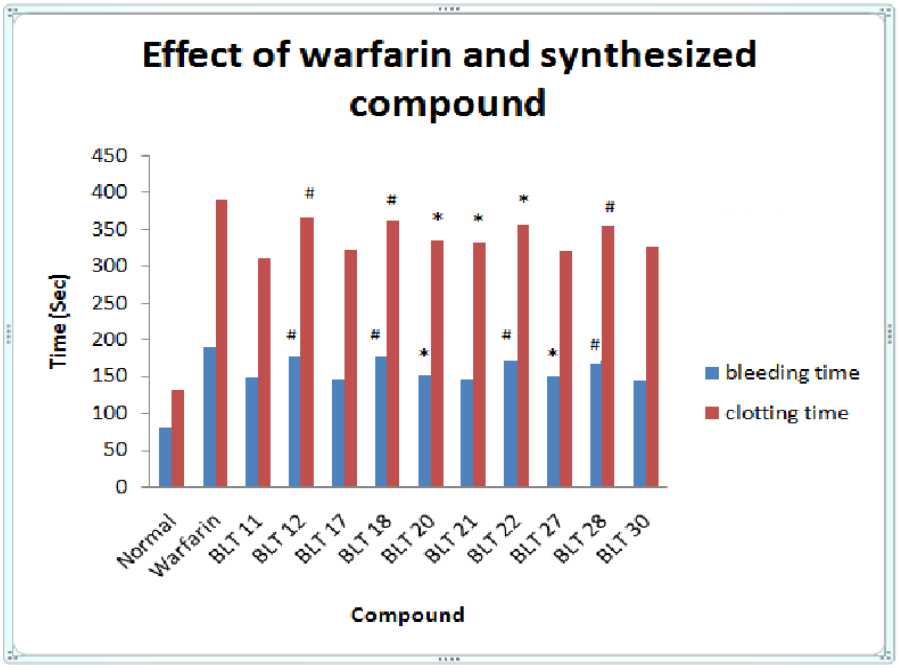
**Tab: 2** Effect of warfarin and synthesized compounds on bleeding and clotting times on rats.

| Blood parameters | Bleeding Time (sec) | Clotting Time (sec) |
|---|---|---|
| Normal | 80+ 12 | 130+ 22 |
| Warfarin | 190+ 18 # | 390+ 35 # |
| BLT 11 | 148 ± 25 | 310 ± 20 |
| BLT 12 | 176 ± 28 # | 365 ± 37 # |
| BLT 17 | 146 ± 37 | 322 ± 43 |
| BLT 18 | 176 ± 43 # | 362 ± 30 # |
| BLT 20 | 152 ± 24 * | 335 ± 52 * |
| BLT 21 | 145 ± 35 | 331 ± 47 * |
| BLT 22 | 170 ± 23 # | 356 ± 52 * |
| BLT 27 | 149 ± 35 * | 320 ± 37 |
| BLT 28 | 171 ± 12 # | 358 ± 29 # |
| BLT 30 | 144 ± 39 | 325 ± 43 |

Values are expressed as Mean + S.E.M

*- significantly different from control ($p < 0.05$), # - significantly different from control ($p < 0.01$)

**Fig: 1** Pharmacological Screening of anticoagulant activity

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**Synthesis of Novel Alkylaminohydroxypropoxy Coumarin Derivatives**

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**ABSTRACT:**

Coumarin molecule is used as anticoagulant. Other uses like anti-HIV, anti-tumor, anti-hypertensive, anti-arrhythmia, anti-osteoporosis, pain relief increase research of coumarin derivative. Perkin discovered a synthetic method of coumarin and opened the door in synthetic research. Now a days there are so many methods are use to form coumarin. Warfarin is a well known drug of 4-hydroxy coumarin derivative. It is consider that, propranolol drug act as a lead molecule of β-blocker. Alkylaminohydroxypropoxy side chain is responsible for β-blocking activity in propranolol. Hence synthesis and it's characterization of antihypertensive coumarin derivative was our main target.

**KEY WORD:**

Coumarin, Anticoagulant, antihypertensive agent, Alkylaminohydroxypropoxy

**INTRODUCTION:**

Coumarin is a widely occurring secondary metabolite that occurs naturally in several plant families and essential oils. Coumarin is an anhydride of o-coumaric acid having white, crystalline lactone, obtainable naturally from several plants, such as tonka

bean, lavender, sweet clover grass, apicots, cherries, strawberries, and cinnamon. It is also synthesized from an amino acid, phenylalanine. [1]

Coumarins are a group of important natural compounds, and have been found to have multi-biological activities such as anti-HIV, anti-tumor, anti-hypertensive, anti-arrhythmia, anti-osteoporosis, pain relief, preventing asthma and antisepsis. [2] Natural products like esculetin, fraxetin, daphnetin and other related coumarin derivatives are recognized as inhibitors not only of the lipoxygenase and cycloxygenase enzymic systems, but also of the neutrophil-dependent superoxide anion generation. Coumarin derivatives also possess anti-inflammatory as well as antioxidant activities. Coumarin possesses immunomodulatory and direct antitumor activity. [3] It has been recommended for treatment of a number of clinical conditions, including high protein oedema and brucellosis. Coumarin and some of its derivatives have been tested for treatment of anxiolytic, microcirculation disorders and angiopathic ulcers, and also for treatment of high protein oedemas in animals. [4]

Coumarin derivatives are used as therapeutic anticoagulants and as rodenticides by causing fatal haemorrhage. Because the range between efficient therapy and undue hemorrhagic risk may vary greatly from one patient to another, the need for carefully individualized treatment and frequent observations has long been stressed. However, a summary of recent research findings, along with certain principles, may offer possible explanations for responsiveness to make highly efficient lead with fewer side effects to resist both, coagulopathy as well as hypertension. The primary aim of this present work is to study pharmacological and synthetic aspects of the coumarin ring structure especially its combined analogues profile as an anticoagulant and antihypertensive property.

The interesting biological properties of coumarin made these compounds very attractive for organic synthesis. Perkin discovers the synthetic method of coumarin and till today thousands of compounds were synthesized. Other named reactions for coumarin synthesis are Pechmann reaction, Knoevenagel reaction, Wittig reaction, Kostanecki-Robinson reaction and Reformatsky reaction. [5] Bose and his colleague discover a new method for synthesis of 4-hydroxy coumarin from substituted phenol. [6]

Warfarin is 4-hydroxy coumarin derivative and used as anticoagulant agent. Warfarin mainly acts via inhibition an enzyme vitamin K epoxide reductase that recycles oxidized vitamin K to its reduced form after it has participated in the carboxylation of several blood coagulation proteins, mainly prothrombin and factor VII. [7] Propranolol is a lead molecule of non-selective β-blockers. It is a derivative of alkylaminohydroxypropoxy side chain, which is responsible for antihypertensive activity. This side chain also affect pharmacokinetic and pharmacodynemic properties of drugs. [8]

The main complications of hypertension, i.e. coronary heart disease, ischemic strokes and peripheral vascular disease are usually related to thrombosis. It therefore seems plausible that use of antithrombotic therapy may be of particular benefit in preventing the thrombosis-related complications of elevated blood pressure. [9] Therefore our aimed was to synthesize coumarin derivatives which may be use as antihypertensive agent.

**RESULT AND DISCUSSION**

Synthesis of following compounds was performed. Coumarin moiety plays important role in anticoagulation of blood and sidechain has antihypertensive property. So following compounds are novel series of coumarin derivatives may be used as antihypertensive agent.

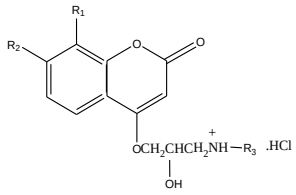


Table.1: Physical characteristics data of synthesized compounds

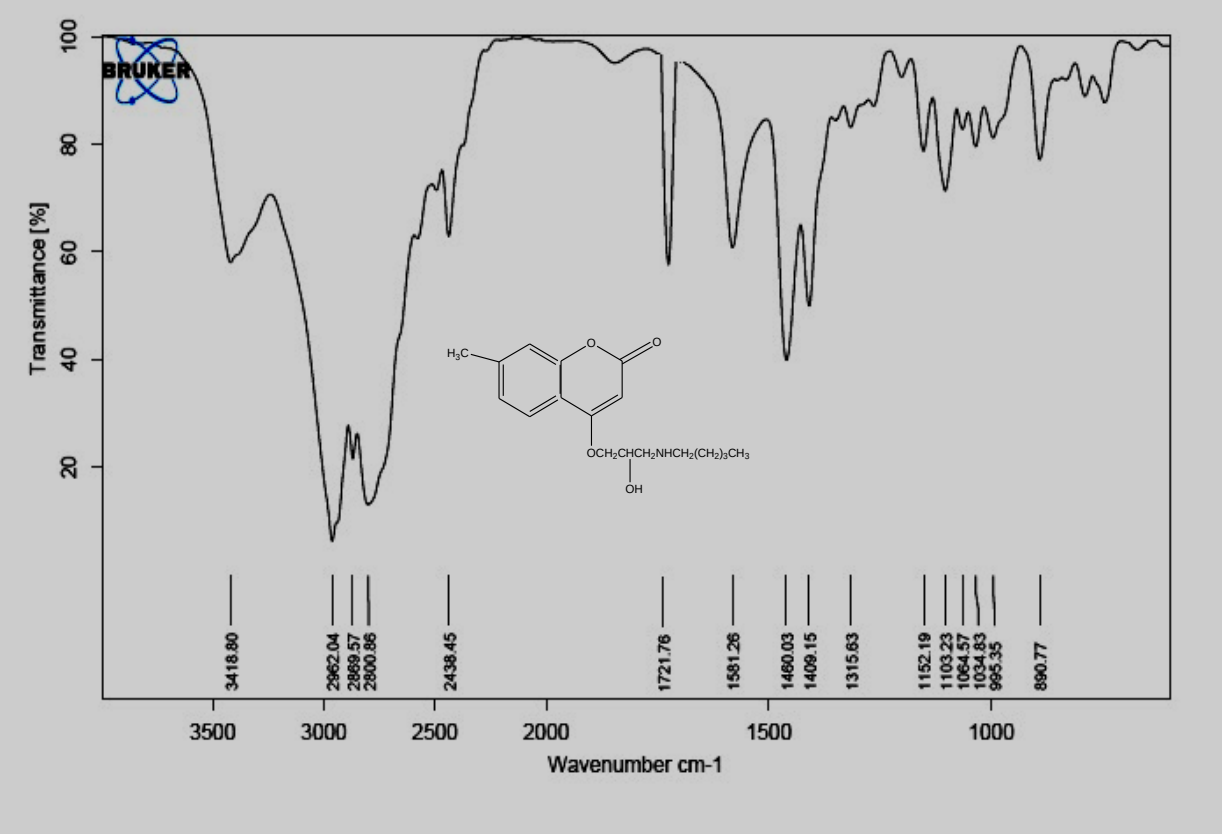
| Sr. No | Code | $R_1$ | $R_2$ | $R_3$ | M.F. | M. P. (°C) | $R_f$ * value | % Yield |
|---|---|---|---|---|---|---|---|---|
| 1 | BLT 1 | H | H | $-CH(CH_3)_2$ | $C_{15}H_{20}NO_4Cl$ | 220-22 | 0.37 | 60 |
| 2 | BLT 2 | H | H | $-C(CH_3)_3$ | $C_{16}H_{22}NO_4Cl$ | 212-14 | 0.35 | 68 |
| 3 | BLT 3 | H | H | $-CH_2(CH_2)_2CH_3$ | $C_{16}H_{22}NO_4$ Cl | 218-20 | 0.33 | 62 |
| 4 | BLT 4 | H | H | $-CH_2(CH_2)_3CH_3$ | $C_{17}H_{24}NO_4$ Cl | 228-30 | 0.33 | 58 |
| 5 | BLT 5 | H | H | $-CH(CH_2)_2$ | $C_{15}H_{18}NO_4$ Cl | 222-24 | 0.33 | 54 |
| 6 | BLT 11 | $-CH_3$ | H | $-CH(CH_3)_2$ | $C_{16}H_{22}NO_4$ Cl | 245-47 | 0.31 | 56 |
| 7 | BLT 12 | $-CH_3$ | H | $-C(CH_3)_3$ | $C_{17}H_{24}NO_4$ Cl | 240-42 | 0.30 | 59 |
| 8 | BLT 13 | $-CH_3$ | H | $-CH_2(CH_2)_2CH_3$ | $C_{17}H_{24}NO_4$ Cl | 247-49 | 0.25 | 61 |
| 9 | BLT 14 | $-CH_3$ | H | $-CH_2(CH_2)_3CH_3$ | $C_{18}H_{26}NO_4$ Cl | 259-61 | 0.27 | 53 |
| 10 | BLT 15 | $-CH_3$ | H | $-CH(CH_2)_2$ | $C_{16}H_{20}NO_4$ Cl | 251-53 | 0.31 | 55 |
| 11 | BLT 21 | H | $-CH_3$ | $-CH(CH_3)_2$ | $C_{16}H_{22}NO_4$ Cl | 251-53 | 0.31 | 50 |
| 12 | BLT 22 | H | $-CH_3$ | $-C(CH_3)_3$ | $C_{17}H_{24}NO_4$ Cl | 244-46 | 0.24 | 48 |
| 13 | BLT 23 | H | $-CH_3$ | $-CH_2(CH_2)_2CH_3$ | $C_{17}H_{24}NO_4$ Cl | 251-53 | 0.28 | 47 |
| 14 | BLT 24 | H | $-CH_3$ | $-CH_2(CH_2)_3CH_3$ | $C_{18}H_{26}NO_4$ Cl | 263-65 | 0.28 | 45 |
| 15 | BLT 25 | H | $-CH_3$ | $-CH(CH_2)_2$ | $C_{16}H_{20}NO_4$ Cl | 257-59 | 0.33 | 46 |

During synthesis many problems were solved by trial and error methods to increase yield. In step one increase the yield by maintain temperature at 65-70 $^0$C. Purification is required in step one, so we tried to purify this products using ethanol: chloroform (9:1) mixture by recrystaliastion method. Epoxy derivatives are formed by using epichlorhydrin in presence of potassium carbonate as a base. At higher temperature sticky products were obtained. Hence this reaction was carried out at 120-125 $^0$C. To get solid product, the epoxy resin was completely separated by using mother solvent i.e. toluene and then dissolved in a minimum quantity of dioxane which was slowly poured in to crushed ice with vigorous stirring and solid epoxy derivative was isolated. Here we have also used benzene in place of toluene but product was not solidified after pour in to crushed ice.

Here epoxy derivative is treated with amine give final product. This reaction was also successful if IPA used as solvent. If solvent or unreacted amine was distilled out then sticky semisolid mass was obtained, so hydrochloric acid salt formation was preferable solution to convert in to solid. This product was hygroscopic and protect from moisture. Synthesized compounds shown in table 1. Here *tert*-butyl amine gives highest yield among this series.

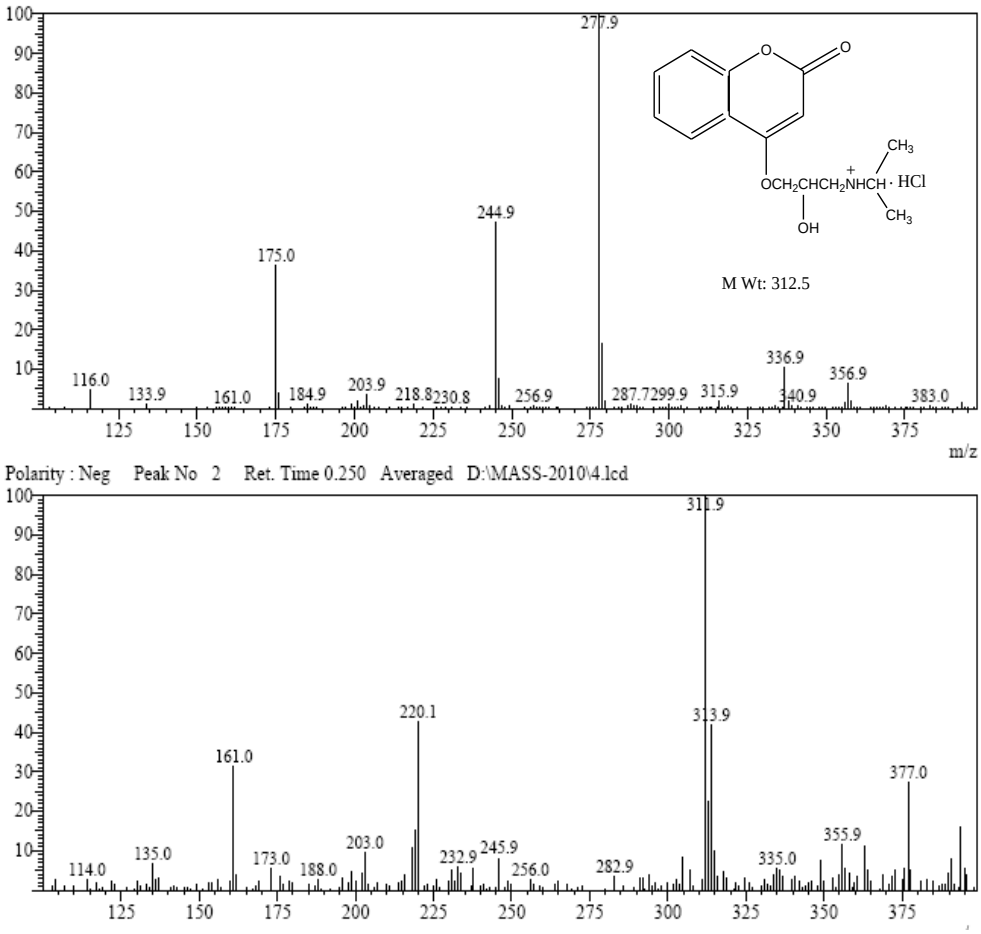
Synthesized compounds were structurally evaluated using spectroscopic methods like, IR, Mass and NMR techniques. In FTIR analysis, a peak was observed at 1700 cm$^{-1}$ of carbonyl group, C-O streching of ring skeleton was observed at 1160-1125 cm$^{-1}$. The N-H streching of secondary amines gives a broad peak between 3350-3300 cm$^{-1}$. The –OH bending observed at 1380-1310 cm$^{-1}$. Other frequencies observed due to ring skeleton are around 1600-1450 cm$^{-1}$ of C=C stretching. Figure 1 show FTIR spectra of BLT2 compounds.

Figure.1: FTIR Spectra of BLT2



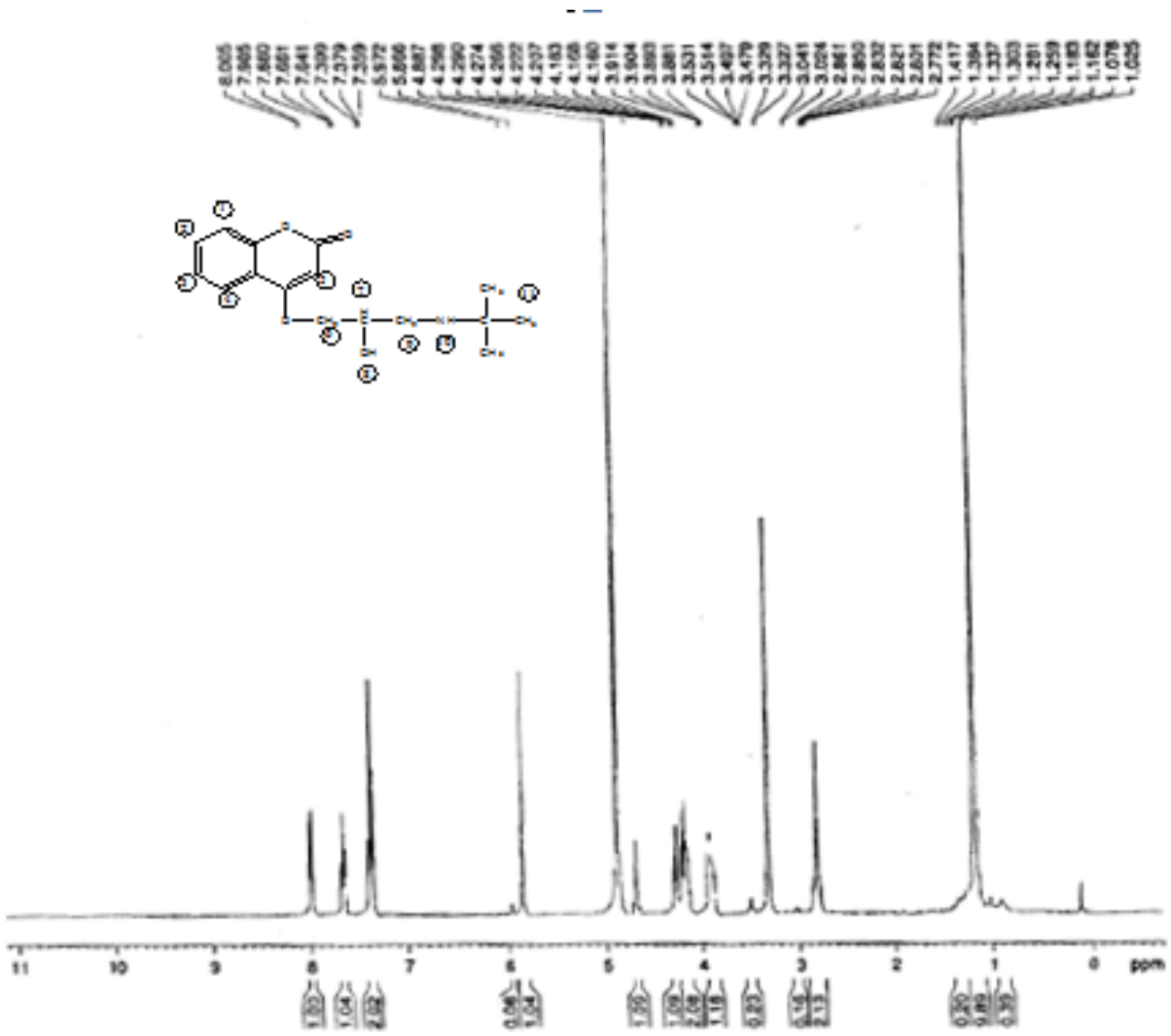

Mass spectra also give structural information of synthesized compound. BLT1 compound was characterized by mass spectra. It shows peak at 311.5 m/z as M-1and other fragments' peak at 219, 175 and 161 m/z. Same fragmentation patterns were observed for all synthesized compounds.

Figure.2: Mass spectra of BLT1




NMR spectra was taken and singlet peaks are observed at 1.16.of $–CH_3$ of sidechain, 3.88 of amine, 5.86 of –OH, 4.88 of $–CH_2$ of ring skeleton, while multiplate, triplate and doublet peaks are also observed.

Figure. 3: NMR Spectra of BLT2



**CONCLUSION:**

Synthesis and structural characterization of novel derivatives, together with the development of new synthetic methods and increase the yield, will be the useful research in thromoembolism and hypertension conditions. It is a first approach to synthesize alkylaminohydroxypropoxy derivative of coumarin. We conclude that *tert*-butyl derivative is formed in higher yield which may posse's antihypertensive and warfarin like activity.

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